Nothing in life is to be feared, it is only to be understood.

- Marie Curie

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Diversity-Oriented Synthesis Based on the Brevianamide Scaffold for the Development of Physiologically Active Compounds

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) of Applied Biological Sciences: Chemistry and Bioprocess Technology

Dutch translation of the title:

Diversiteitsgebaseerde synthese van brevianamide analogen voor de ontwikkeling van fysiologisch actieve componenten

Please cite as:

I. Wauters, 'Diversity-oriented synthesis based on the brevianamide scaffold for the development of physiologically active compounds', PhD dissertation, Ghent University, 2015

Cover illustration:

Asexual spores from the *Aspergillus* mold. Photo credit: Özlem Sarikaya-Bayram

This research was funded by the Research Foundation-Flanders (FWO Vlaanderen)

ISBN number: 978-90-5989-833-2

EAN barcode:

9 789059 898332 >

Ghent, October 2015

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List of abbreviations

AA	amino acid
ACH	acetone cyanohydrin
ACN	acetonitrile
AIBN	azobisisobutyronitrile
AMP	4-(aminomethyl)piperidine
APCI	atmospheric pressure chemical ionization
aq.	aqueous
ATP	adenosine triphosphate
ATR	attenuated total reflectance
BBN	9-borabicyclo[3.3.1]nonane
BCRP	breast cancer resistance protein
Boc	*tert*-butyloxycarbonyl
BOPCl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
Bt	benzotriazol-1-yl
BtH	benzotriazole
Cbz	benzyloxycarbonyl or carboxybenzyl
CCD	charge-coupled device
CCDC	Cambridge crystallographic data center
CDI	1,1'-carbonyldiimidazole
cGMP	cyclic guanosine monophosphate
Cosy	correlation spectroscopy
Cpd	compound

CV	column volume
DAPI	4’,6-diamidino-2-phenylindole
dba	dibenzylideneacetone
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DFT	density functional theory
DIPEA	ethyldiisopropylamine
DKP	diketopiperazine or piperazin-2,5-dione
DMAP	4-dimethylaminopyridine
DMAPP	dimethylallylpyrophosphate
DMDO	dimethyldioxirane
DMF	*N*,*N*-dimethyl formamide
DMSO	dimethyl sulfoxide
dr	diastereomeric ratio
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess
ESI	electrospray ionization
FAD	flavin adenine dinucleotide
Fmoc	fluorenylmethoxycarbonyl
Gly	glycine
GMP	guanosine monophosphate
GTP	guanosine triphosphate

H2BC	heteronuclear 2-bond correlation spectroscopy
HFIP	hexafluoroisopropanol
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMPA	hexamethylphosphoramide
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HSQC	heteronuclear single-quantum correlation spectroscopy
Hyp	(2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid
IC	inhibitory concentration
IL	ionic liquid
IR	infrared
LDA	lithium diisopropylamide
MAP	microtubule-associated proteins
mCPBA	*meta*-chloroperoxybenzoic acid
MDR	multi drug resistance
MEM	methoxyethoxymethyl
MIC	minimal inhibitory concentration
Ms	mesyl or methanesulfonyl
MS	mass spectrometry
MSDS	material safety data sheet
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide
MW	microwave
NBS	*N*-bromosuccinimide

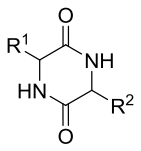
NCS	*N*-chlorosuccinimide
NMM	*N*-methylmorpholine
NMMO	*N*-methylmorpholine *N*-oxide
NMP	*N*-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
on.	overnight (ca. 16 h)
OS	olefin strain
PMB	*para*-methoxybenzyl
ppm	part per million
Pg	protecting group
PPi	pyrophosphate
Pro	proline
pTLC	preparative thin layer chromatography
pTsOH	*para*-toluenesulfonic acid
Quant.	quantitative
r.t.	room temperature (19-25 °C)
RT	residence time
SAR	structure–activity relationship
SD	standard deviation
T	temperature
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl

TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOF	time-of-flight
Troc	2,2,2-trichloroethoxycarbonyl
Trp	tryptophan
Trt	trityl or triphenylmethyl
Ts	tosyl or 4-toluenesulfonyl
UV-VIS	ultraviolet-visible
VWD	variable wavelength detector

I. Introduction and goals

Natural products and their derivatives are very often used as lead compounds for the development of biologically active molecules.[1] The percentage of medicinal compounds derived from natural products is about 25%.[2-3] A wide plethora of natural products exist, despite the limited amount of building blocks used by organisms to synthesize their primary and secondary metabolites. An example of a commonly used class of building blocks are the amino acids, which can be linked to form proteins or can be transformed into complex secondary metabolites *via* specialized synthetic pathways.

When two amino acids undergo a double condensation, cyclic dipeptides **1** are formed, which are known as piperazin-2,5-diones or 2,5-diketopiperazines (DKPs). These are the smallest cyclic peptides (Figure 1). These compounds were recognized as a separate class of compounds around 1900. The first synthetic diketopiperazine, cyclo(Gly, Gly), was made in 1888[4] and many of the simpler members of this class were synthesized soon thereafter.[5]



1

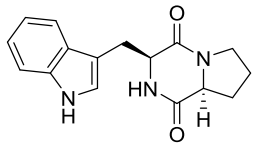
Figure 1: General 2,5-diketopiperazine structure 1.

2,5-Diketopiperazines were originally found in hydrolysates of proteins and processed food and drinks, where they can influence the flavor.[6] Therefore, these compounds were considered to be side products from terminal proteolysis during fermentation and food processing. However, since then they have been recognized as secondary metabolites. They are mostly present in fungi, but they can also be found in animals and plants. New additions to the family are commonly isolated from cultures of fungi.

The 2,5-diketopiperazines possess several properties that make them attractive as a scaffold for medicinal chemistry. The restricted conformational freedom as a result of their cyclic structure endows them with several benefits when compared to their linear dipeptide counterparts. Most importantly they are more resistant to enzymatic degradation by proteases with respect to linear dipeptides, which results in a longer preservation of their biological activity. Diketopiperazines can be used as scaffolds to synthesize peptidomimetic drugs with enhanced activity. Moreover, their constrained structure can result in higher receptor selectivity or higher binding affinities in their interaction with receptors. Their rigid structure can also be used to determine the active conformation of a protein.[7]

The class of 2,5-diketopiperazines is thus promising for the development of new drugs. The number of possible cyclic dipeptides is rather extensive. Using merely the 20 most abundant L-amino acids already gives rise to 200 different diketopiperazines, but there is no limitation to the use of D-isomers or other unnatural amino acids. So, where to start?

Through evolution, conservation of certain structural features has arisen among natural products, which results in overrepresentation of a limited number of scaffolds in a large number of different secondary metabolite classes.[8] These entities entail specific biological activities and can be considered 'privileged (sub)structures'.[9] Their presence often endows the molecule with a high affinity for its receptor, and the same privileged scaffold can do so for parent molecules targeting totally unrelated receptors.[10] These privileged (sub)structures are a good indication to look for interesting scaffolds.[11] An example is the cyclo(L-Trp, L-Pro) scaffold, also known as brevianamide F **2a** (Figure 2). This structural unit, which displays biological activity on its own, can be found in many fungal secondary metabolites with a variety of activities.



2a

Figure 2: Brevianamide F 2a.

Scaffold **2a** constitutes the core structure in the families of the brevianamides (**2a**, **3**) (antibacterial),[12] austamides (**4**),[13-14] spirotryprostatins (**5**) (cell cycle inhibitor),[15] tryprostatins (**6**) (microtubule inhibitor),[16-17] fumitremorgins (**7**) (BCRP inhibitor),[18] versicolamides (**8**),[19] paraherquamides (**9**) (antihelminthic, insecticidal),[20-21] sclerotamides (**10**),[22-23] notoamides (**11**),[24-26] avrainvillamides (**12**),[27] malbrancheamides (**13**) (calmodulin inhibitor),[28-29] stephacidins (**14**) (antitumor)[30], aspergamides (**16**)[31] and VM55599 (**15**) (Figure 3). These are all fungal secondary metabolites that are produced by different *Aspergillus* and *Penicillium* species.

The amino acids that make up the cyclic dipeptide will influence the activity of the final compound. Proline differs from the other amino acids because of its secondary amino group that is embedded in a cyclic sidechain. When it forms an amide bond it can no longer act as a hydrogen bond donor, only as a hydrogen bond acceptor. The cyclic sidechain also influences the conformation and promotes the cyclization tendency of dipeptides.

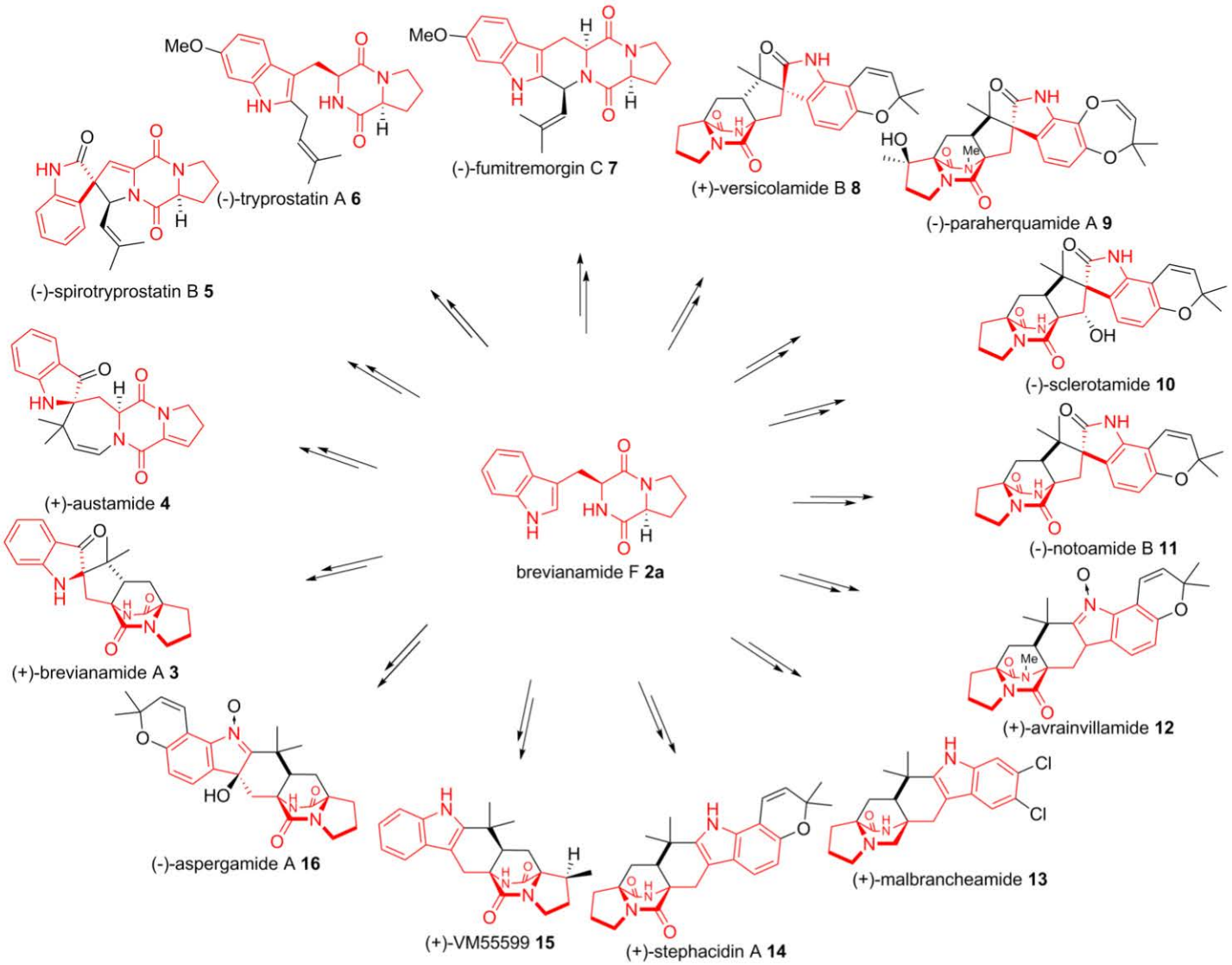


Figure 3: Fungal secondary metabolites made up of cyclo(L-Trp, L-Pro) 2a.

Tryptophan possesses the indole moiety, a structural unit which is associated with several bioactive compounds like the neurotransmitter serotonin **17**, the hormone melatonin **18**, and the auxins, plant hormones like indol-3-ylacetic acid **19** (Figure 4).[32]

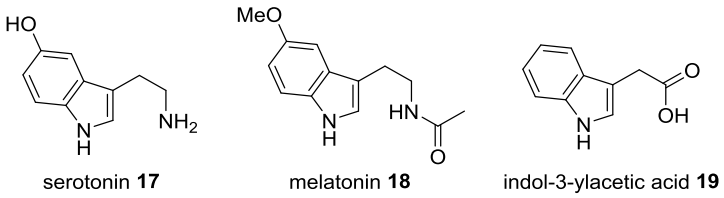


Figure 4: Indoles with important activity in animals and plants.

The natural products in Figure 3 mostly have a multifunctionalized and rather complex diketopiperazine skeleton. In view of the broad range of their biological activities (anticancer, antibacterial, insecticidal), it is very appealing to study synthetic analogues with a less complex structure. Scaffold **2** can be seen as the key intermediate towards these different natural products. Therefore, it was chosen as the starting material in the present research. The aim was to decorate the basic skeleton **2** through the application of various synthetic methodologies to generate novel analogues.

The first part of the research will focus on the synthesis of the basic skeleton **2** from the amino acids tryptophan and proline. These two amino acids frequently occur in natural diketopiperazines (Figure 3). Different synthetic strategies will be evaluated, after which the basic skeleton will be used for the synthesis of analogues with a diverse substitution pattern.

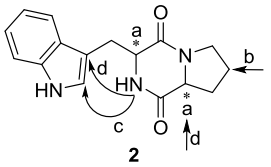
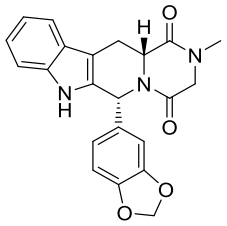


Figure 5: Possible sites of modification in the cyclo(Trp, Pro) scaffold 2.

The arrows in Figure 5 show the different positions of scaffold **2**, which can be modified using straightforward synthetic methodologies. The use of amino acids with an opposite chirality will lead to the study of the activity of the unnatural counterparts (Figure 5, modification a). Natural diketopiperazines are primarily made up from natural L-amino acids. Consequently, they are chiral molecules that mainly exist in the *cis*-configuration. Nonetheless, some piperazin-2,5-diones consisting of D-amino acids have been found to occur naturally.[7, 33] Moreover, tadalafil **20**, a commercially available diketopiperazine-based drug for the treatment of erectile dysfunction, also contains the unnatural D-isomer of tryptophan (Figure 6).[34] Thus, the incorporation of unnatural amino acids does not exclude the possibility of displaying biological activity.



20

Figure 6: Tadalafil 20.

The use of hydroxyproline units (Figure 5, modification b) will allow for the decoration of the basic scaffold later on in the synthesis, through the introduction of substituents important in medicinal chemistry (heterocyclic nuclei, fluorine containing substituents, morpholines, cyclopropyl substituents). In this way, starting from a basic diketopiperazine scaffold, a diverse set of analogues may be prepared in a highly efficient, divergent way.

From the basic scaffold **2**, different strategies can be adopted to prepare annulated (Figure 5, modification c) as well as spiro-derivatives (Figure 5, modification d) with different ring sizes. The introduction of a supplementary ring in the basic skeleton will enhance the conformational rigidity of the scaffold, which may lead to increased receptor specificity. In research towards active analogues from natural products, conformational restriction is often evaluated.[35-39]

Next to the synthesis of the compounds, attention will be paid to the testing of their bioactivity. Compounds will be evaluated in collaboration with the department of Radiotherapy and Experimental Cancerology (UZ Gent), the Laboratory of Microbiology (UGent) or by Cerep (France).

II. Literature overview

1. Introduction

The 2,5-diketopiperazines are abundantly present in nature.[33, 40-42] The most widespread and structurally diverse 2,5-diketopiperazine subclass is made up from the amino acids tryptophan and proline. Several modes of (iso)prenylation of the simple cyclic dipeptide precursor cyclo(L-Trp, L-Pro) or brevianamide F **2a** lead to the development of a diverse family of diketopiperazines. Further heterocyclization of nonannulated compounds has resulted in the formation of annulated and spiro-annulated diketopiperazines, while some compounds possess a more complex diazabicyclo[2.2.2]octane core.[42]

The brevianamides are a group of mould metabolites and were the first tryptophan-proline based diketopiperazines to be isolated. Over the years numerous new compounds have been found (Figure 3). This literature study focuses on the brevianamides, which include nonannulated, annulated and bridged diazabicyclo[2.2.2]octane-based products. Additionally, the (spiro- and cyclo-)tryprostatins and fumitremorgins are discussed, because of their interesting bioactivity, which makes them promising leads for anticancer drugs.

2. Brevianamides

The brevianamides A-F are secondary metabolites that were originally isolated by Birch *et al*. in 1969 from the mould *Penicillium brevi-compactum*, and were the first examples of this new class of fungal alkaloids.[43-45] The structures of brevianamide A-F are shown in Figure 7.

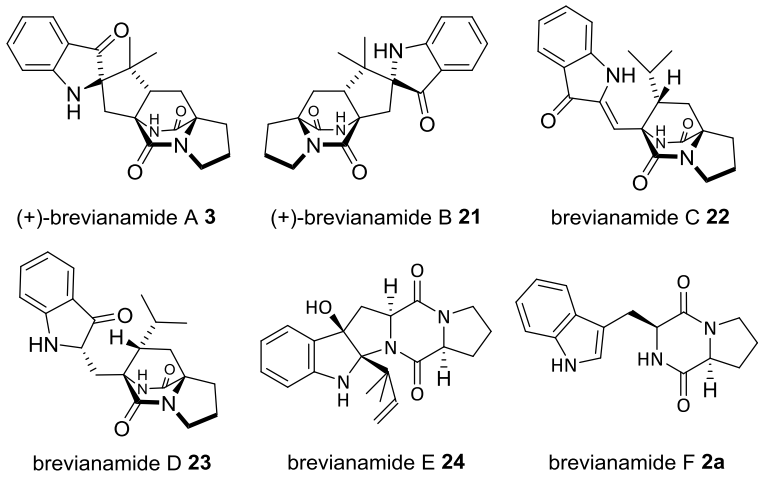
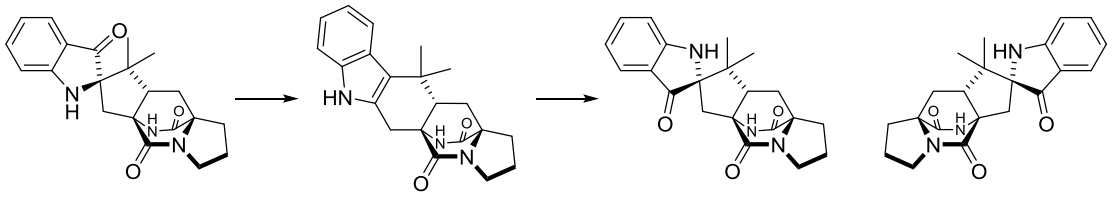


Figure 7: Structures of brevianamides A-F.

Brevianamide A **3** was the most abundant of the reported metabolites, isolated from *Penicillium brevi-compactum*. Its relative and absolute stereochemistry were established through X-ray crystallographical analysis of its 5-bromo derivative.[46] In addition, brevianamide A **3** was also found in other cultures, such as *Penicillium viridicatum*[47] and *Penicillium ochraceum*.[48]

Brevianamide A **3** failed to display antibiotic or antifungal properties.[47] However, it possesses antifeedant and insecticidal activities.[49] The compound was evaluated against *Drosophila melanogaster* for feeding inhibition and against *Spodoptora littoralis* for feeding inhibition and mortality. At a concentration of 27 nM, it displayed a significant effect in all three assays. Similar antifeedant activity was detected against *Spodoptera frugiperda* and *Heliothis virescens*.[50] Brevianamide A **3** is thus a mycopesticide (a pesticide produced by fungi). Preliminary toxicity studies of brevianamide A **3** showed no acute toxicity in mice upon oral or intraperitoneal administration. However, the molecule induced cytotoxicity and inflammatory lung response in intratracheally exposed mice, which prohibits its application as an insecticide in food crops.[51-52]

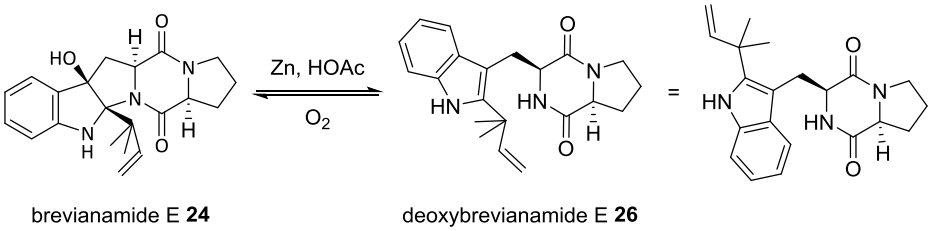
Brevianamide B **21** is a diastereomer of brevianamide A **3**, with all centres but the spirocarbon inverted. They are enantiomorphs with respect to the diazabicyclo[2.2.2]octane ring.[53] The natural occurring (+)-brevianamide A **3** can be interconverted into the unnatural (-)-enantiomer of brevianamide B **21** (Scheme 1). Reduction of brevianamide A **3** with sodium borohydride and acidic dehydration of the resulting hydroxyindoline with concomitant rearrangement results in deoxybrevianamide A **25**.[44] Subsequent re-oxidation to the hydroxyindolenine and rearrangement with base yields (-)-brevianamide B **21**.[45] Brevianamide A and B are found to appear after conidiation and are mainly present in the aerial hyphae of the fungus.[54-55]



Scheme 1: Interconversion of (+)-brevianamide A 3 in (-)-brevianamide B 21.

Characterization studies revealed that brevianamides C **22** and D **23** are in fact photochemical artifacts formed during fermentation and isolation, and not true metabolites of enzymatic origin. Both can be prepared by irradiation of brevianamide A **3** and they were not detected in *P. brevi-compactum* when grown in the dark.[45]

The absolute configuration of brevianamide E **24** was determined on the basis of its first enantioselective total synthesis.[56-57] Reduction of brevianamide E **24** with zinc in acetic acid gave deoxybrevianamide E **26** (Scheme 2).[43-44] Deoxybrevianamide E **26** has been isolated as a metabolite in cultures of *Aspergillus ustus* and is a natural product on its own.[13, 58] Deoxybrevianamide E **26** slowly converts into brevianamide E **24** by aerial oxidation, leading to the suspicion of brevianamide E **24** being an artifact.[59] However, if this should be the case, brevianamide E **24** would have been detected in the cultures of *A. ustus* alongside of deoxybrevianamide E **26**. Since no trace of brevianamide E **24** was present, the compound cannot be an artifact, but represents a dead end in the biosynthetic pathway.[60]



brevianamide E **24** deoxybrevianamide E **26**

Scheme 2: Relation between brevianamide E 24 and deoxybrevianamide E 26.

Brevianamide F **2a**, the simplest of the brevianamides, is the condensation product of L-tryptophan and L-proline. Its biological activity has been studied most extensively. Preliminary investigations have shown the potential of cyclo(L-Trp, L-Pro) **2a** as an antimicrobial substance.

Compound **2a** also showed potential for use in the treatment of cardiovascular dysfunction, since it affected sodium, calcium and potassium channels.[61] The effects of all four isomers of **2** on the heart and ion-channel activity were examined (Table 1).

Cyclo(D-Trp, L-Pro) **2b** displayed an antagonistic effect on the calcium channel thus inhibiting the influx of Ca^{2+} (blocking the current), while the other isomers acted as agonists increasing the inward current of Ca^{2+}. All four isomers exhibited antagonistic effects on the sodium ion channel. No effect was noted on the inward rectifier potassium current for cyclo(L-Trp, D-Pro) **2c** and cyclo(D-Trp, D-Pro) **2d**, while cyclo(L-Trp, L-Pro) **2a** and cyclo(D-Trp, L-Pro) **2b** behaved as antagonists.

Table 1: Qualitative representation of the effects of the isomers on Ca^{2+}-, K^+-, Na^+-channel activity.

Cyclic dipeptide	Effect on:		
	Ca^{2+} channels[a]	**Na^+ channels**[b]	**K^+ channels**[c]
	100 µM	*100 µM*	*10 µM*
Cyclo(L-Trp, L-Pro) **2a**	agonist	antagonist	antagonist
Cyclo(D-Trp, L-Pro) **2b**	antagonist	antagonist	antagonist
Cyclo(L-Trp, D-Pro) **2c**	agonist	antagonist	no significant effect
Cyclo(D-Trp, D-Pro) **2d**	agonist	antagonist	no significant effect

[a] Holding potential -90 mV and test potential 5 mV. [b] Holding potential -90 mV and test potential -30 mV. [c] Holding potential -80 mV and test potential between -140 mV and -50 mV.

The *cis*-isomers (**2a** and **2d**) showed no significant effect on the heart rate. In contrast, cyclo(L-Trp, D-Pro) **2c** increased the heart rate and cyclo(D-Trp, L-Pro) **2b** exhibited a decrease in heart rate. The coronary flow rate was not influenced by any of the isomers. In addition, all isomers significantly reduced the duration of ventricular tachycardia (rapid heartbeat) and arrhythmia (irregular heartbeat, too fast or too slow), as well as the time to sinus rhythm (normal heartrate) thus showing antiarrhythmic potential.[62] However, further studies revealed the hepatotoxicity of the isomers.[63] The administration of the compounds involves potential harm, which may limit their potential usage in the treatment of various diseases.

Also antifungal activity has been recorded.[64] Moreover, brevianamide F **2a** gave potent inhibition of seedling growth. A possible explanation for this behavior lies in the structural similarity between brevianamide F **2a** and indol-3-ylacetic acid **19**, a plant hormone involved in plant growth and development. Brevianamide F **2a** might block the interaction of indol-3-ylacetic acid **19** with its auxin receptor, resulting in plant growth inhibition. Brevianamide F **2a** might lead the way to a natural, eco-friendly herbicide.[65]

Later studies have reported on the isolation of new members of the brevianamide family such as brevianamide J **27**, a dimer, and brevianamide K **28**. Both were isolated from the fungus *Aspergillus versicolor*.[66] Brevianamides Q **29** and R **30** were also obtained from the solid-state fermented rice culture of the fungus *Aspergillus versicolor* (Figure 8).[67] Several other diketopiperazine alkaloids were isolated, namely brevianamides L-P. In contrast to the earlier discussed members of the brevianamide class, these metabolites do not bear a cyclo(Trp, Pro) core.

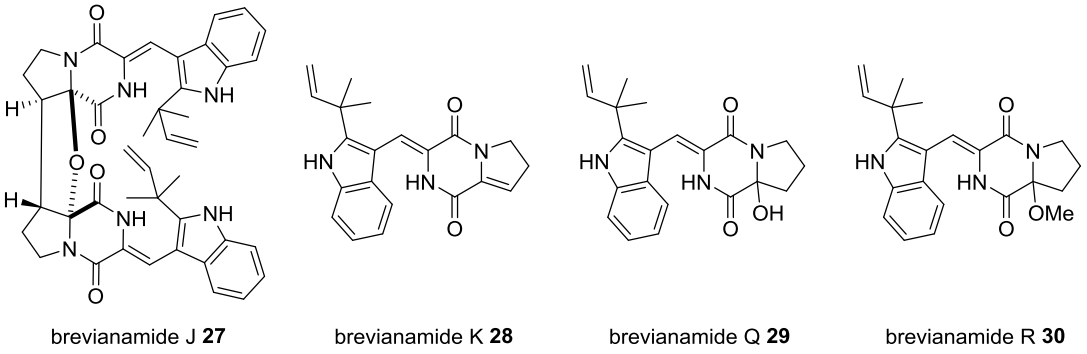


Figure 8: Structures of brevianamides J, K, Q and R.

The latest addition to the brevianamide series are the brevianamides S-V (Figure 9).[68] These alkaloids were found in an *Aspergillus versicolor* isolated from sediment obtained in the Bohai Sea, China. Brevianamide S **31** displayed no significant inhibitory activity against several Gram-positive and -negative bacteria and the fungus *Candida albicans*. However, a significant antibacterial acivity against Bacille Calmette-Guérin was reported. Selectivity towards the latter, a screening surrogate for *Mycobacterium tuberculosis*, is indicative of a new mechanism of action and might thus constitute a very appealing hit in the search for new antitubercular drugs. Brevianamide J **27** and brevianamide S **31** are the only two hitherto discovered brevianamides, which are members of the class of dimeric diketopiperazines.

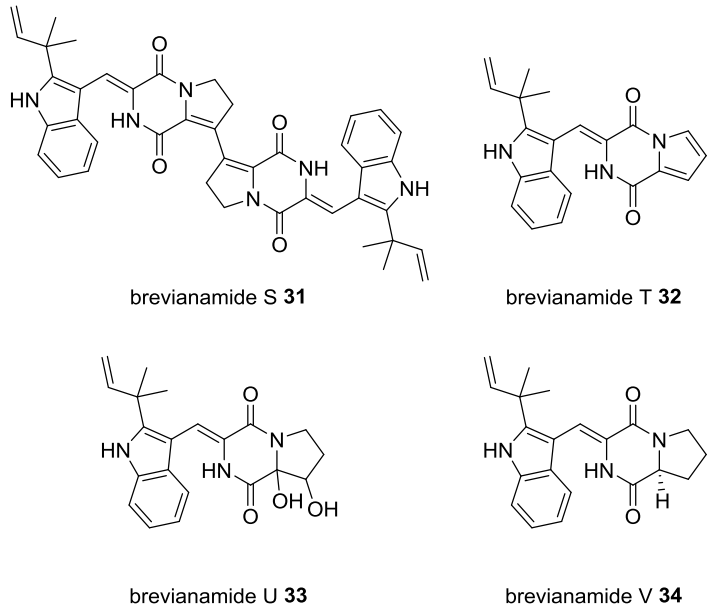


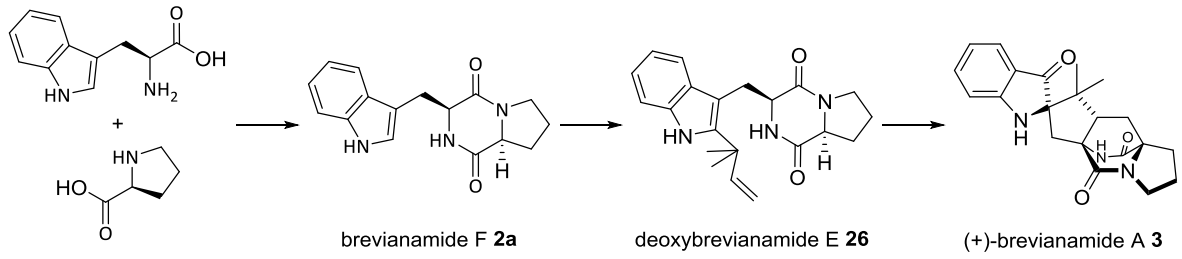
Figure 9: Brevianamides S-V.

3. Biosynthetic pathway

In the following part, the current knowledge on the biosynthesis of brevianamides A **3** and B **21** will be discussed. Their structural complexity arises from the presence of both the spirocyclic junction and the bicyclic structure, and several mechanisms to their origin have been proposed.

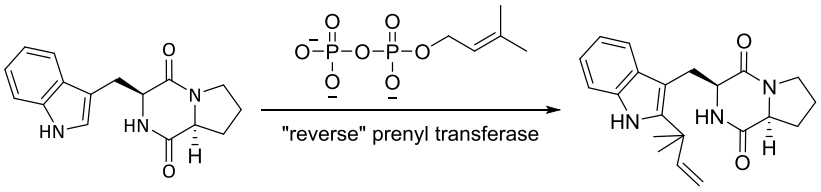
During the structural elucidation of brevianamide A **3**, the presence of an indole group and a diketopiperazine ring suggested tryptophan as a biological precursor, with a link to another amino acid. Moreover, mass and NMR spectra indicated the presence of an isoprene unit. Feeding experiments with ^{14}C-labeled tryptophan, proline and mevalonic acid lactone resulted in significant incorporation into brevianamide A **3** by *P. brevi-compactum*, confirming that brevianamide A **3** may be biosynthetically derived from proline, tryptophan and mevalonic acid. On the basis of this work, a biosynthetic precursor in the form of deoxybrevianamide E **26** was suggested.[44] Additionally, a feeding experiment with ^{14}C-labeled cyclo(L-Trp, L-Pro) **2a** proved that brevianamide F **2a** is incorporated into brevianamide A **3**.[69]

As a result, a biosynthetic sequence was proposed starting with the coupling of the amino acids L-proline and L-tryptophan. A gene encoding for a brevianamide F synthetase enzyme has been identified.[70] Brevianamide F **2a** is subsequently converted in brevianamide A **3** *via* the intermediate deoxybrevianamide E **26** (Scheme 3).[69]



Scheme 3: Biosynthetic pathway to brevianamide A 3.

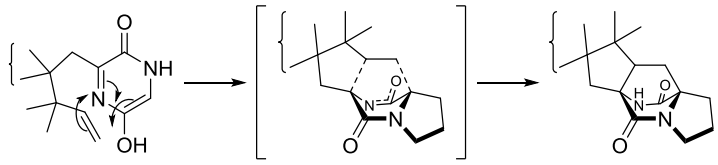
Radio-labeled [8-^{3}H]deoxybrevianamide E **26** was used to validate the hypothesis that this molecule is an intermediate in the biosynthetic pathway of brevianamides A **3**, B **21** and E **24**.[60] The formation of deoxybrevianamide E **26** from brevianamide F **2a** necessitates the introduction of an isoprenyl group at the C-2 position of the indole (Scheme 4). This isoprene unit is delivered by dimethylallylpyrophosphate (DMAPP), arising from the mevalonate pathway. The most recent report for the attachment of the isoprenyl group suggests the presence of a "reverse" prenyl transferase in which DMAPP undergoes electrophilic attack at C-3 *via* an S_N2' mechanism instead of at the phosphorylated end.[71]



brevianamide F **2a** deoxybrevianamide E **26**

Scheme 4: Introduction of the isoprene unit.

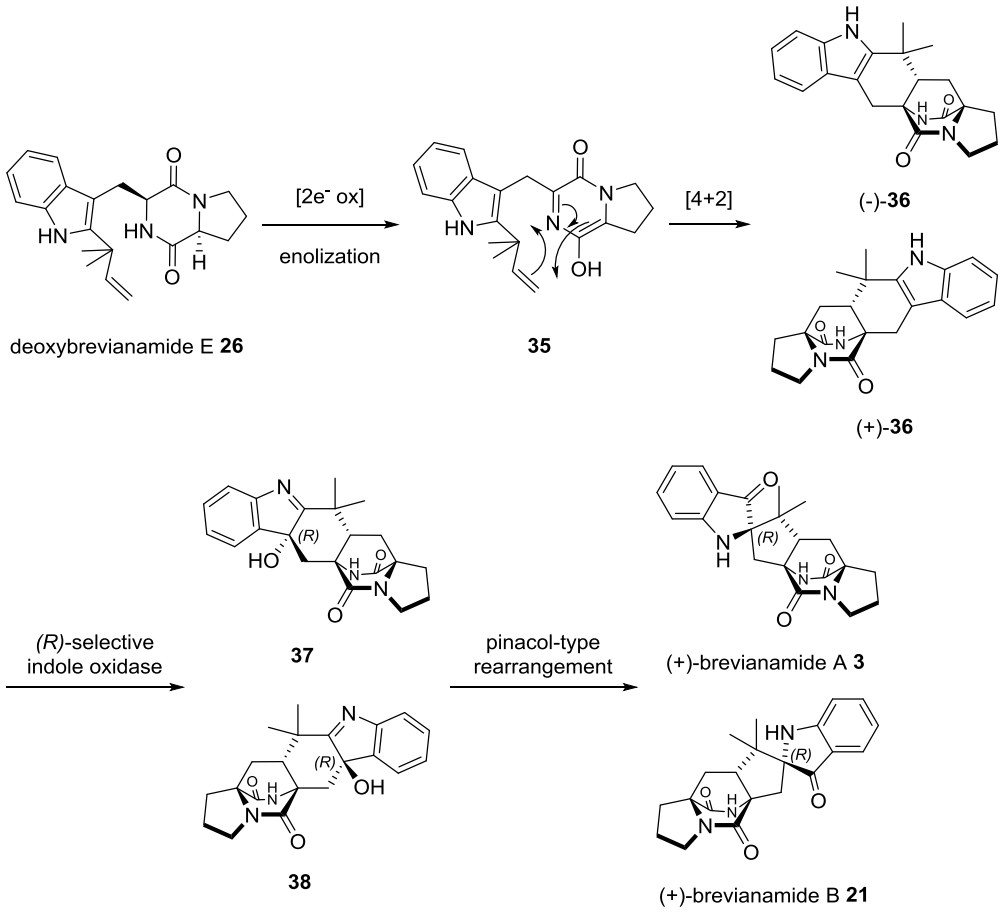
The most intriguing feature is the diazabicyclo[2.2.2]octane core present in (among others) brevianamide A **3** and B **21**. Porter and Sammes postulated that the bridged architecture was the result of an intramolecular Diels-Alder reaction (Scheme 5).[72]. Such a [4+2] cycloaddition reaction has rarely been proven to occur in the biosynthesis of natural products. Hence, it is not surprising that the biosynthetic origin of the tricyclic framework has received a lot of attention.[23, 60, 73-80] Two possible routes for the transformation of deoxybrevianamide E **26** into brevianamide A **3** and B **21** have been investigated. The two pathways differ in the order in which the intramolecular Diels-Alder reaction and the formation of the spiro-indoxyl group take place.



Scheme 5: Proposed biosynthetic Diels-Alder reaction constructing the bicyclo[2.2.2] ring system.

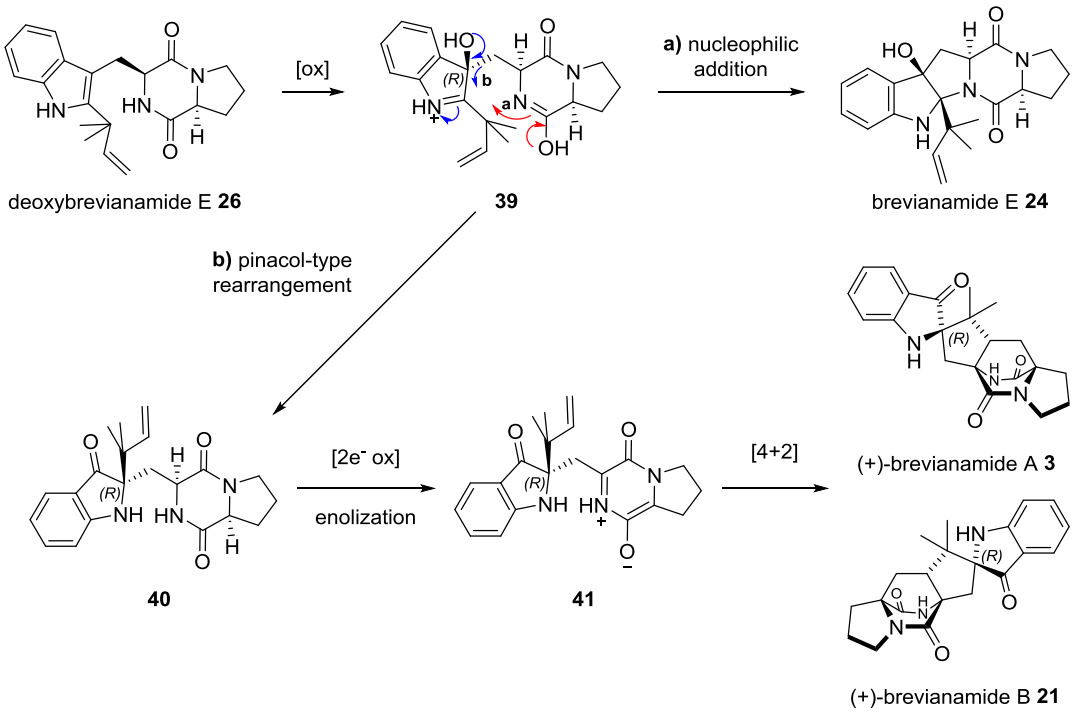
The original biosynthetic proposal included the formation of the achiral azadiene **35** by the oxidation of the tryptophan unit and enolization of the proline unit from deoxybrevianamide E **26** (Scheme 6). Cycloaddition of **35** with the isoprenyl group can take place at both sides of the azadiene, resulting in a mixture of hexacyclic compounds **36**. Taking into account that brevianamide A **3** and B **21** are produced by the fungi in different amounts, this would imply the formation of the enantiomers **36** in unequal amounts. Since compound **36** does not autoxidize to either brevianamide A **3** and B **21**, this would imply an enzyme-mediated conversion of **36** into **3** or **21**. A hydroxyl group would be introduced, proposedly by an (*R*)-selective indole oxidase. Then, a pinacol-type ring contraction of the hydroxyindolenines **37**and **38** would lead to brevianamides A **3** and B **21**.

The main concern with this proposal was that the most abundant metabolite, brevianamide A **3**, would be formed by oxidation at the most hindered side of the indole group (*via* **37**), while the minor metabolite, brevianamide B **21**, would arise from oxidation at the least hindered side of **36**.[53, 81] When feeding experiments proved that structures **36** are not intermediates in the biosynthesis of brevianamides A **3** and B **21**, this pathway was abandoned.[60]



Scheme 6: Original proposal for the biogenesis of brevianamide A 3 and B 21.

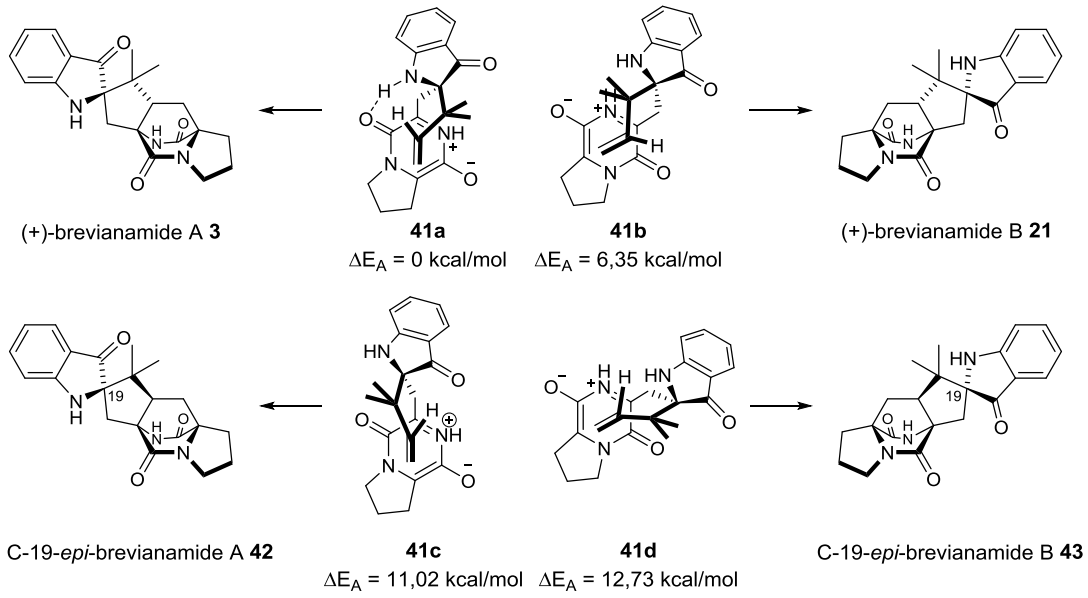
Subsequently, a different biosynthetic pathway was proposed that also takes into account the formation of brevianamide E **24** (Scheme 7).[60, 74] In this proposal, deoxybrevianamide E **26** undergoes an (*R*)-selective hydroxylation reaction at C-3 of the indole unit, furnishing 3-hydroxyindolenine **39**. Thereupon, compound **39** is either converted in brevianamide E **24** *via* an irreversible nucleophilic addition of the secondary amide-N to the iminium bond of **39** (pathway a) or, alternatively, *via* a stereospecific pinacol-type rearrangement of 3-hydroxyindolenine **39** (pathway b) that affords an (*R*)-stereochemistry at the quaternary spiro-carbon of **40**. The latter is subsequently transformed in brevianamide A **3** and B **21** through the oxidation and enolization of the diketopiperazine core (**41**) and an intramolecular Diels-Alder reaction.



Scheme 7: Current proposed biosynthetic route for brevianamides A, B and E.

The transition state structures of the last step of this pathway (**41**) were further investigated to assess the possibility of a biological Diels-Alder reaction, during which the azadiene **41** would undergo a [4+2] cycloaddition with the isoprenyl group (Scheme 8). A priori, four diastereomers can be formed, but only (+)-brevianamide A **3** and B **21** are known natural products. The relative activation energies of the four stereoisomeric transition state structures **41** were calculated relative to **41a** and are depicted in Scheme 8. Structures **41a** and **41b** lead to the formation of brevianamide A **3** and B **21**, respectively, while structures **41c** and **41d** would lead to **42** and **43**, diastereomers of **3** and **21**, which are not known to occur in nature.[75]

These calculations are made assuming compounds are in the gas phase, in the absence of solvent. This is in contrast to the natural, aqueous environment of the cells. It is known that a polar solvent like water can enhance the Diels-Alder reaction.[82] So these *ab initio* studies do not accurately mimick the biological environment. However, the findings concur with the observations and are helpful to rationalize what's happening.



Scheme 8: The calculated relative energies for transition state structures 41 of the intramolecular [4+2] cycloaddition.

The transition state structure for brevianamide A (**41a**) has the lowest potential energy barrier, which can be explained by the formation of an intramolecular hydrogen bond. This stabilizes the transition structure resulting in the major formation of brevianamide A **3** as the most abundant secondary metabolite in contrast to brevianamide B **21**.[75] It has been shown that the intramolecular cycloaddition in these systems can proceed spontaneously at room temperature.[77] The potential energy barriers towards stereoisomers **42** and **43** is much higher, accordingly these compounds (presumably) do not occur in nature. Their higher activation energy is caused by the orientation of the vinyl group with respect to the azadiene, which is in turn influenced by the concomitant formation of the five-membered ring during the Diels-Alder reaction (Figure 10). This forces the vinyl group to twist around the forming C-C bonds 1-2 and 3-4. As a consequence these three bonds (1-2, 2-3 and 3-4) are not in the same plane. The dihydral angle (1-2-3-4) is bigger for the transition structures leading to **42** and **43** than the one in the intermediates leading to brevianamides A **3** and B **21**. This leads to an ineffective overlap in the structures **41c** and **41d** explaining their higher activation energies.

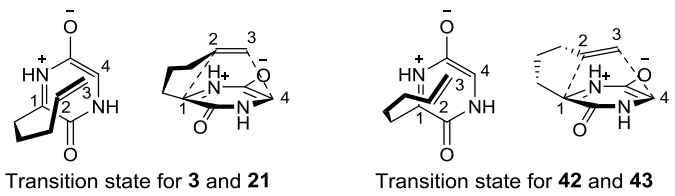
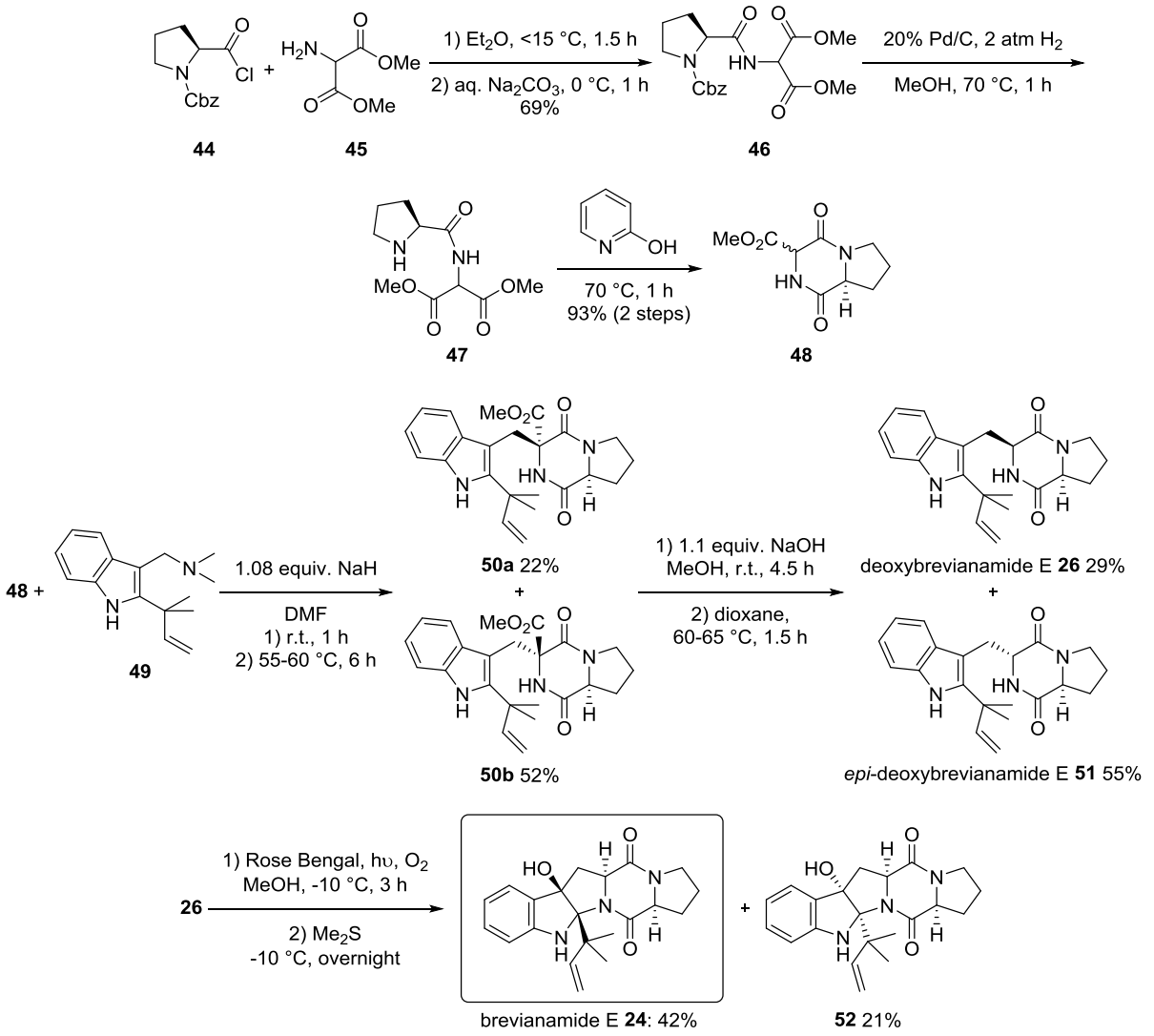


Figure 10: Top and front view of the transition state structures to 3, 21, 42 and 43.

4. Syntheses

The first asymmetric total synthesis of brevianamide E **24** was reported by Kametani *et al.* in 1980 (Scheme 9).[56-57] This total synthesis of brevianamide E was the first one in the brevianamide series to be reported. This was partly due to the simpler structure of brevianamide E **24** with respect to that of brevianamide A **3** and B **21**, and partly because its structure had not yet been confirmed by X-ray crystallography at the time, in contrast to brevianamide A **3**.[59]

The diketopiperazine core was synthesized starting from the acid chloride **44** of *N*-Cbz-L-proline, which was reacted with dimethyl 2-aminomalonate **45** under Schotten-Baumann conditions to give **46**. Deprotection of the Cbz group by hydrogenolysis yielded the free amine **47**, which was heated in the presence of hydroxypyridine to afford the diketopiperazine **48**. Condensation of **48** with isoprenylated gramine **49** using sodium hydride provided two diastereomers **50a** and **50b**. Both **50a** and **50b** were subsequently converted to deoxybrevianamide E **26** and its epimer **51** by hydrolysis and decarboxylation of **50**. In the final step, deoxybrevianamide E **26** was subjected to photo-oxidation in the presence of Rose Bengal followed by treatment with dimethyl sulfide, furnishing brevianamide E **24** and its diastereomer **52**.

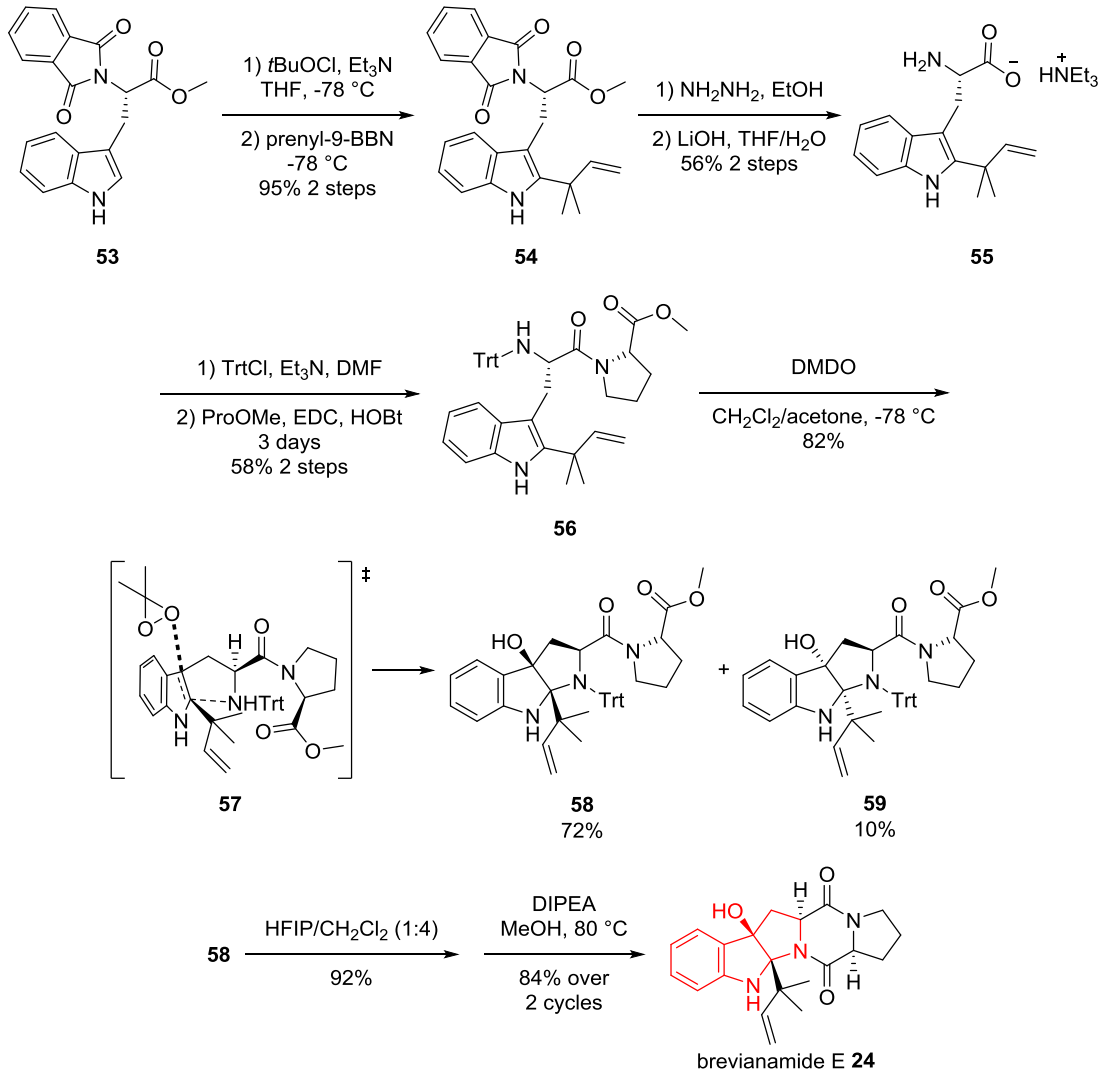


Scheme 9: Asymmetric total synthesis of brevianamide E 24.

The synthesis of brevianamide E **24** has been studied by several groups over the past 35 years.[56-57, 59-60, 83-84] The main challenge comprised the synthesis of the *syn-cis* configured hydroxypyrroloindolenine core (marked in red in **24**, Scheme 10). The majority of the developed syntheses were based on an oxidative cyclization step using photo-oxidation (*vide supra*) or dimethyldioxirane (DMDO)[84] of deoxybrevianamide E **26**, which always resulted in a mixture of diastereomers: *syn-cis* brevianamide E **24** and *anti-cis epi*-brevianamide E **52**.

The first stereoselective synthesis of brevianamide E **24** dates from 2012 (Scheme 10).[85] An isoprenyl group was introduced at C-2 of the indole in N^{α}-phthaloyl-tryptophan methyl ester **53** using prenyl-9-BBN. Removal of the phthaloyl and methyl groups from **54** under basic conditions gave the triethylammonium salt **55** after workup. Subsequent tritylation of **55** and coupling of proline methyl ester under standard conditions yielded dipeptide **56**. The key step in the sequence was the selective DMDO-mediated oxidation of dipeptide **56**, which yielded the *syn-cis* diastereomer **58** in high

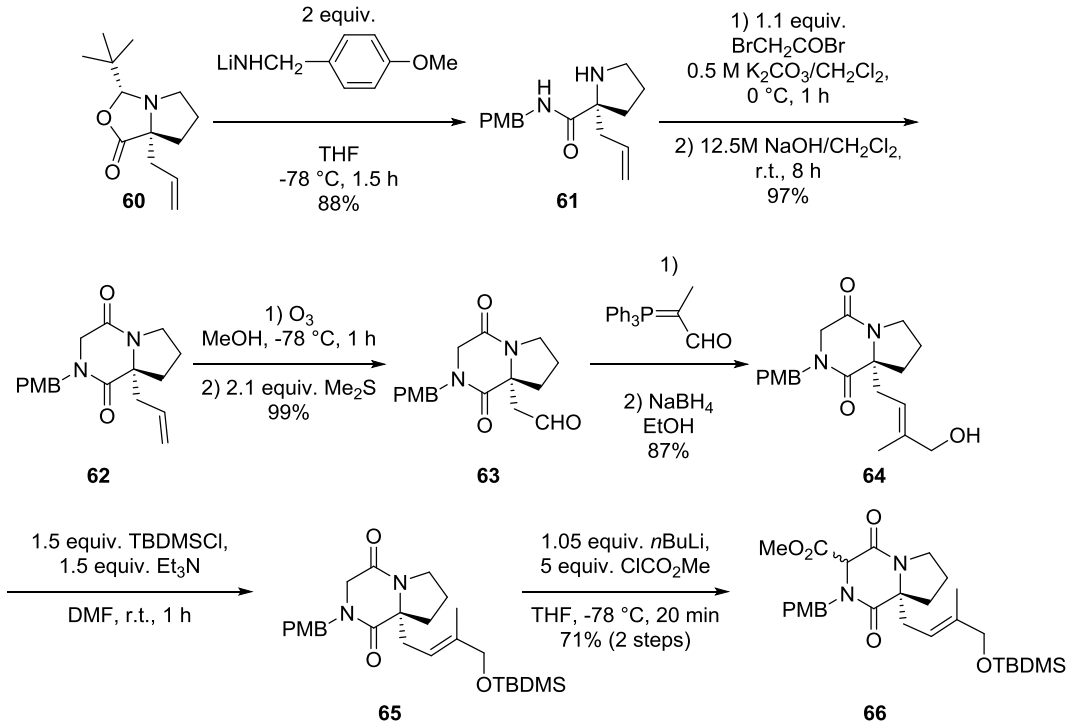
selectivity (yield 82%, *dr* > 8:1). This contrasts with the poor diastereoselectivities obtained in DMDO-mediated oxidations of various other dipeptides of the form *N*–Trt-Trp-AA-OMe (where AA is one of 12 standard amino acids). The increased steric bulk of proline compared to other amino acids favors the formation of one diastereomeric transition state **57**, in which DMDO-mediated oxidation and pyrrole ring closure are synchronized to favor the *syn-cis* product **58**. The presence of the nitrogen from Trp may further promote the facial indole reactivity. Removal of the trityl group in the presence of hexafluoroisopropanol (HFIP) and treatment with DIPEA gave brevianamide E **24**.



Scheme 10: Stereoselective synthesis of brevianamide E 24.

The structure of brevianamide A **3** had already been confirmed by X-ray analysis of its 5-bromo derivative.[46] Brevianamide B **21** was thought to be the stereoisomer of brevianamide A **3** about the spiro-centre.[45] The first total synthesis of brevianamide B **21** did not only confirm its structure,[86] but also revealed that both the synthetic brevianamide B and the semisynthetic product derived from brevianamide A **3** were of the opposite absolute configuration of natural (+)-brevianamide B **21**.[53, 81]

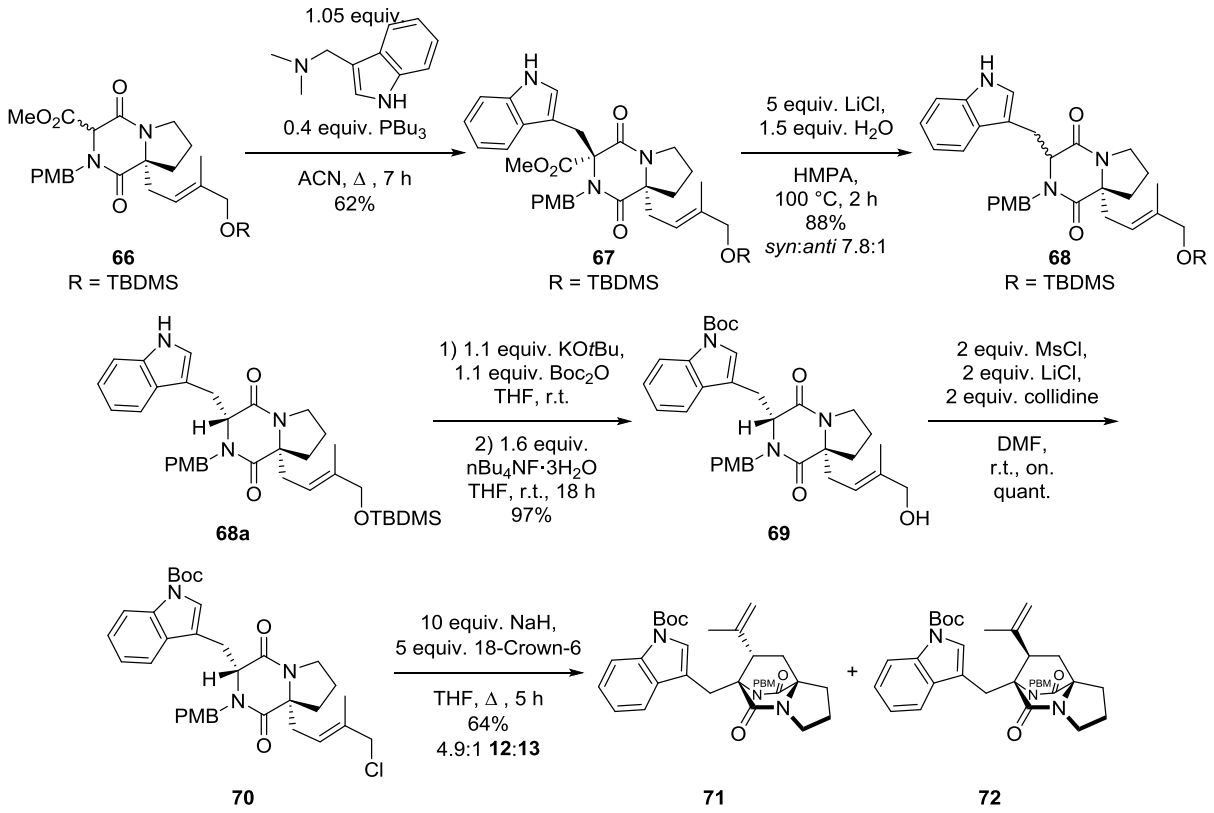
This synthesis started from allylated proline derivative **60**, which was reacted with lithium *p*-methoxybenzylamine to form the amide **61**. Condensation of **61** with bromoacetyl bromide and subsequent ring closure yielded DKP **62**. Ozonolysis of **62** furnished aldehyde **63**, which was converted into allylic alcohol **64** by a Wittig reaction and a reduction. Allylic alcohol **64** was protected as the corresponding *tert*-butyldimethylsilyl ether **65**. Next, methoxycarbonylation of **65** afforded a diastereomeric mixture of **66** (Scheme 11).



Scheme 11: Synthesis of intermediate 66 towards (-)-brevianamide B 21.

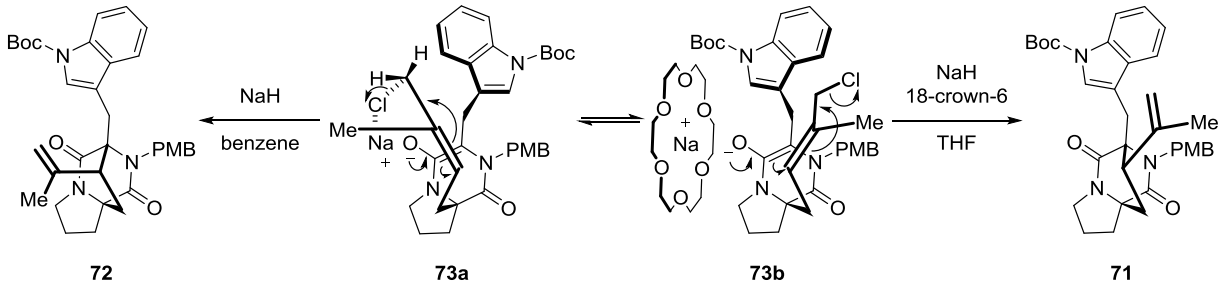
Following the methodology of Kametani the mixture of diastereomers **66** was treated with gramine and a single diastereomer **67** was obtained (Scheme 12).[56, 87] Demethoxycarbonylation of **67** involved hydrolysis to the carboxylic acid and rapid thermal decarboxylation to furnish **68** with the desired *syn*-isomer **68a** as the main product. Compound **69** was obtained by protection of the indole-N and deprotection of the allylic alcohol of **68a**. Conversion of allylic alcohol **69** into the allylic chloride **70** was followed by an intramolecular S_N2' cyclization to **71** and **72**. This step is crucial for

setting the stereogenic center at the bridge. It was found that the cyclization of **70** in the presence of sodium hydride and 18-crown-6 ether resulted in the formation of mainly the desired isomer **71** (in a 4.9:1 ratio) while the reaction performed with sodium hydride in benzene resulted in the highly stereoselective formation of the undesired isomer **72** in 82% yield (**71**:**72** 3:97 ratio).



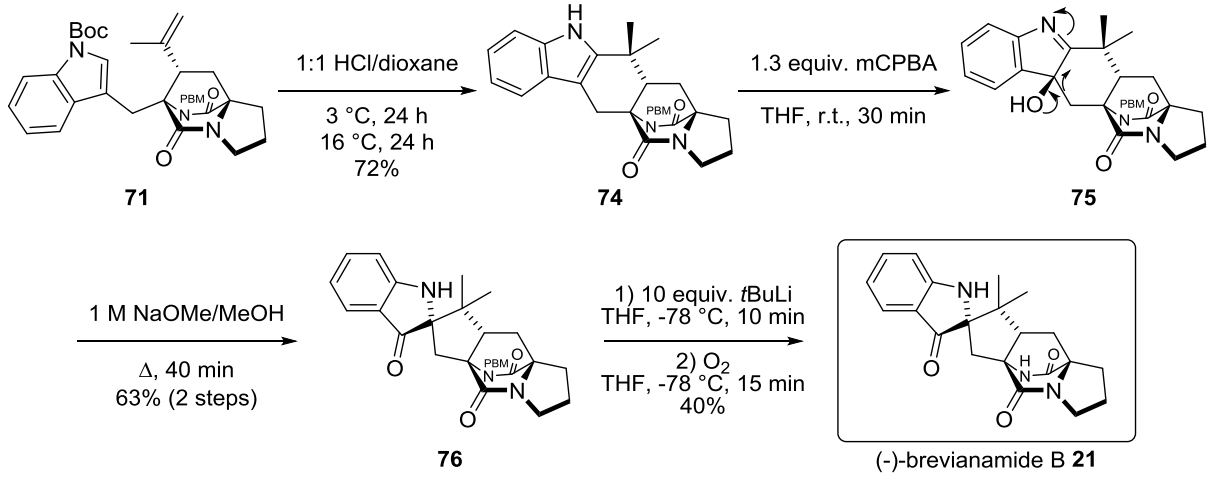
Scheme 12: Synthesis of intermediate 71 towards (-)-brevianamide B 21.

The rationalization for this change in stereoselectivity is based on the assumption that the environment of the enolate that is generated during the reaction plays an important role in the formation of the two possible products **71** and **72** (Scheme 13). In good cation-solvating conditions a bulky solvent shell can be created around the sodium cation. This creates a sterically demanding environment around the enolate oxygen. As a result, the allylic chloride will fold over the enolate as depicted in transition state **73b** to obtain the correct transition-state geometry for cyclization. However, cyclization in the nonpolar, poor ligating solvent benzene favors the formation of transition state **73a**, which brings the sodium cation and the developing chloride anion in close proximity, resulting in the formation of **72**.



Scheme 13: Rationalization for the change in stereochemistry leading to 71 and 72.

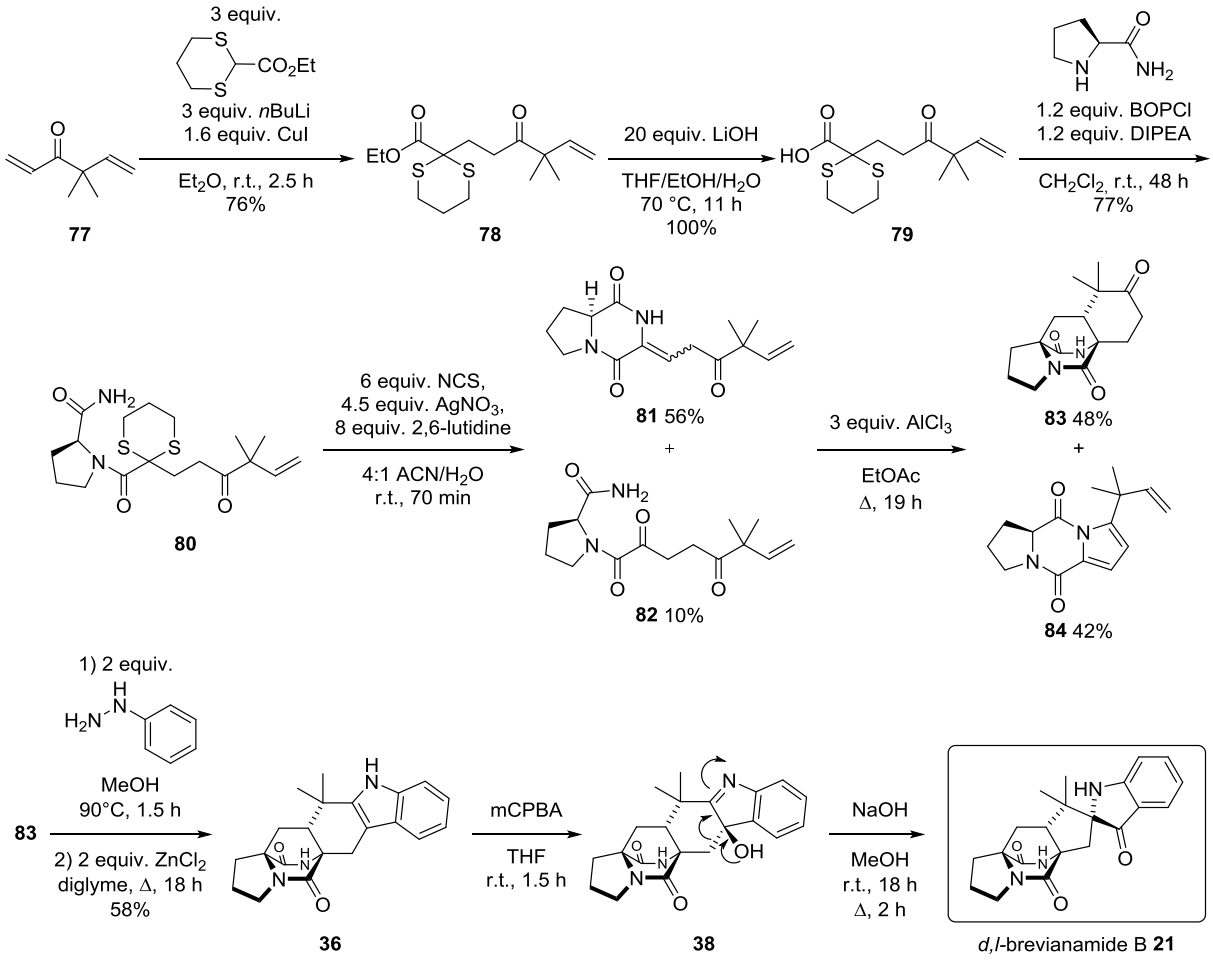
To complete the synthesis of (-)-**21**, compound **71** was stirred with HCl in dioxane to effect concomitant removal of the N-Boc protecting group and cyclization to produce the hexacycle **74** (Scheme 14). Oxidation of **74** with mCPBA at the least hindered side of the indole moiety led to hydroxy indolenine **75**, which after a base-induced rearrangement furnished indoxyl **76**. The final step in the synthesis of (-)-brevianamide **21** involved the removal of the *p*-methoxybenzyl group, which required deprotonation with *tert*-butyllithium and quenching of the benzylic anion with oxygen.



Scheme 14: Final steps in the synthesis of (-)-brevianamide B 21.

A shorter, diastereoselective synthesis of *d*,*l*-brevianamide B **21** has also been accomplished by the group of Williams (Scheme 15).[88] A different approach for the formation of the tricyclic core was used here. Michael addition of ethyl 1,3-dithiane-2-carboxylate to dienone **77** gave dithiane **78**. Hydrolysis of the ester provided acid **79**, which was coupled with L-prolinamide to compound **80**. Oxidative deprotection of **80** gave a mixture of diketopiperazine **81** and **82**. The mixture of **81** and **82** was treated with $AlCl_3$ leading to the desired enantiomeric Diels-Alder adducts **83** and side product **84**. After isolation of the enantiomers **83,** the indole heterocycle was formed *via* a Fischer indole reaction. The final conversion of the 2,3-disubstituted indole **36** to the spiro-indoxyl moiety was similar to the final steps in the previously described synthesis of (-)-brevianamide B **21**, including the

stereoselective epoxidation to 3-hydroxyindolenine **38** and pinacol-type rearrangement to form racemic brevianamide B **21**.



Scheme 15: Synthesis of a mixture of brevianamide B enantiomers.

5. Other Trp-Pro based diketopiperazines

A family of prenylated Trp-Pro-based diketopiperazines has been isolated from the fermentation broth of the marine fungus *Aspergillus fumigatus* BM939 by Osada *et al.* These compounds include tryprostatin A, B,[15, 89-90] the cyclotryprostatins A-D,[91] spirotryprostatin A and B,[92-93] fumitremorgin C and demethoxyfumitremorgin C,[15] all of which cause cell cycle arrest at the G2/M phase (Table 2).

Table 2: Inhibitory activity towards the cell cycle progression of tsFT210 cells.

Cpd		IC_{50} (µM)	MIC (µM)
6	Tryprostatin A	78.7	16.4
85	Tryprostatin B	18.8	4.4
86	Spirotryprostatin A	197.5	
5	Spirotryprostatin B	14.0	
87	Cyclotryprostatin A	5.6	
88	Cyclotryprostatin B	19.5	
89	Cyclotryprostatin C	23.4	
90	Cyclotryprostatin D	25.3	
7	Fumitremorgin C	14.0	4.1
91	Demethoxyfumitremorgin C	**1.78**	**0.45**

5.1. Tryprostatins

Tryprostatin A **6** and B **85** arrest cell cycle progression at the G2/M phase in tsFT210 cells (a cell line used to detect inhibitors of the mammalian cell cycle) with IC_{50} values of 78.7 µM for **6** and 18.8 µM for **85** (Figure 11, Table 2).[15, 89-90] Tryprostatin A **6** inhibits the formation of microtubules, which are essential for mitosis, by interfering with the interaction between microtubule-associated proteins (MAP) and the C-terminal domain of tubulin.[16, 94] This represents a novel mechanism, as most other antimitotic agents interact with tubulin.[95]

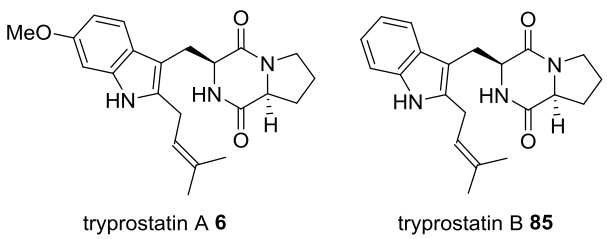
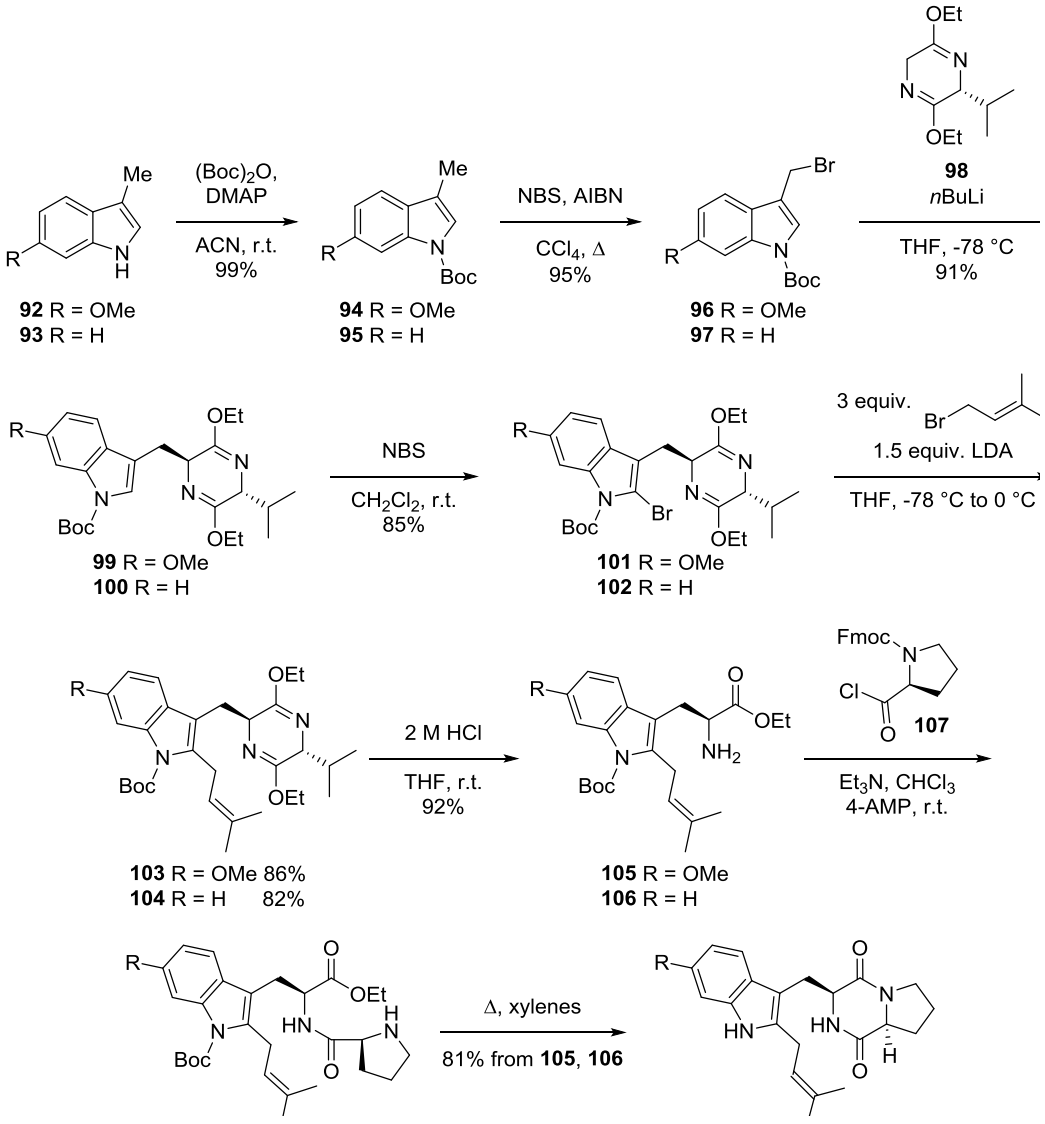


Figure 11: Tryprostatin A 6 and B 85.

Moreover, tryprostatin A **6** was inhibits the breast cancer resistance protein (BCRP).[17] BCRP is an energy-dependent (ATP) transmembrane drug-efflux pump, which contributes to the multidrug resistance (MDR) of cancers. An increased expression of BCRP causes resistance of certain cancer cell lines against antitumor drugs like mitoxantrone, topotecan and doxorubicin, by preventing the chemotherapeutic drugs from reaching lethal levels within the cells. Inhibitors of BCRP can thus be considered chemosensitizers. In normal tissues the expression of BCRP is limited, which makes inhibition of BCRP an attractive strategy to reverse MDR. Tryprostatin A **6** reverses a mitoxantrone-resistant phenotype in the human gastric carcinoma cell line EPG85-257RNOV and the human breast cancer cell line MCF-7/AdrVp (both cell lines exhibited acquired BCRP-mediated MDR) at concentrations of 10–50 µM, so that the cellular mitoxantrone accumulation is no longer BCRP-dependent. At these concentrations no cytotoxicity was observed.[17] The presence of the methoxy group in tryprostatin A **6** reduces the cytotoxicity compared to tryprostatin B **85** and enhances the specificity for inhibition of microtubule assembly. A MTT assay determined that the IC_{50} of tryprostatin A **6** was 400 µM whereas tryprostatin B **85** displayed an IC_{50} value of 4 µM.[96] Tryprostatin A analogues may thus be considered a hit in the search for novel antimitotic and BCRP inhibiting agents.

Despite their rather simple, nonannulated structure, the synthesis of **6** and **85** requires several steps to obtain the appropriately substituted tryptophan derivatives **105** and **106**.[97-98] In a sequence reported by Cook *et al.*, indoles **92** and **93** are protected by the introduction of a Boc group (Scheme 16). Then, the protected 3-methylindoles **94** and **95** are reacted with NBS to afford the 3-(bromomethyl)indoles **96** and **97**. Reaction of brominated compounds with the anion of the Schöllkopf chiral auxiliary **98** provides the desired *trans*-diastereomers **99** and **100** with 100% diastereoselectivity. Next, using NBS, bromination of the indole C-2 position is accomplished. The resulting compounds **101** and **102** are treated with LDA and prenyl bromide to introduce the prenyl group. Hydrolysis of the dihydropyrazine unit in **103** and **104** under acidic conditions affords the substituted L-tryptophan derivatives **105** and **106**. The coupling of the latter with *N*-Fmoc-L-prolyl chloride **107**, together with removal of the Fmoc group, is accomplished by the addition of the base 4-(aminomethyl)piperidine (4-AMP). Finally, the dipeptides **108** and **109** are heated in xylenes at reflux to yield tryprostatins A **6** and B **85**.



Scheme 16: Enantiospecific total synthesis of tryprostatin A 6 and B 85.

5.2. Spirotryprostatins

The spirotryprostatins A **86** and B **5** possess a unique spiro-fusion to a pyrrolidine at the 3-position of the oxindole (Figure 12). This intriguing feature is characteristic for the spirotryprostatins and is not present in the brevianamide series. The spirotryprostatin A **86** displayed the weakest biological activity of the DKP family of cell cycle inhibitors (Table 2).

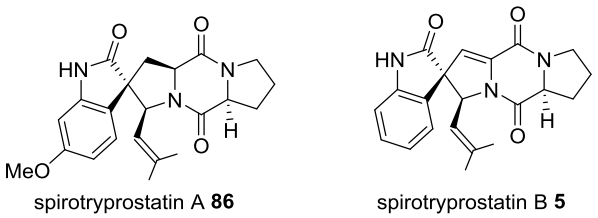
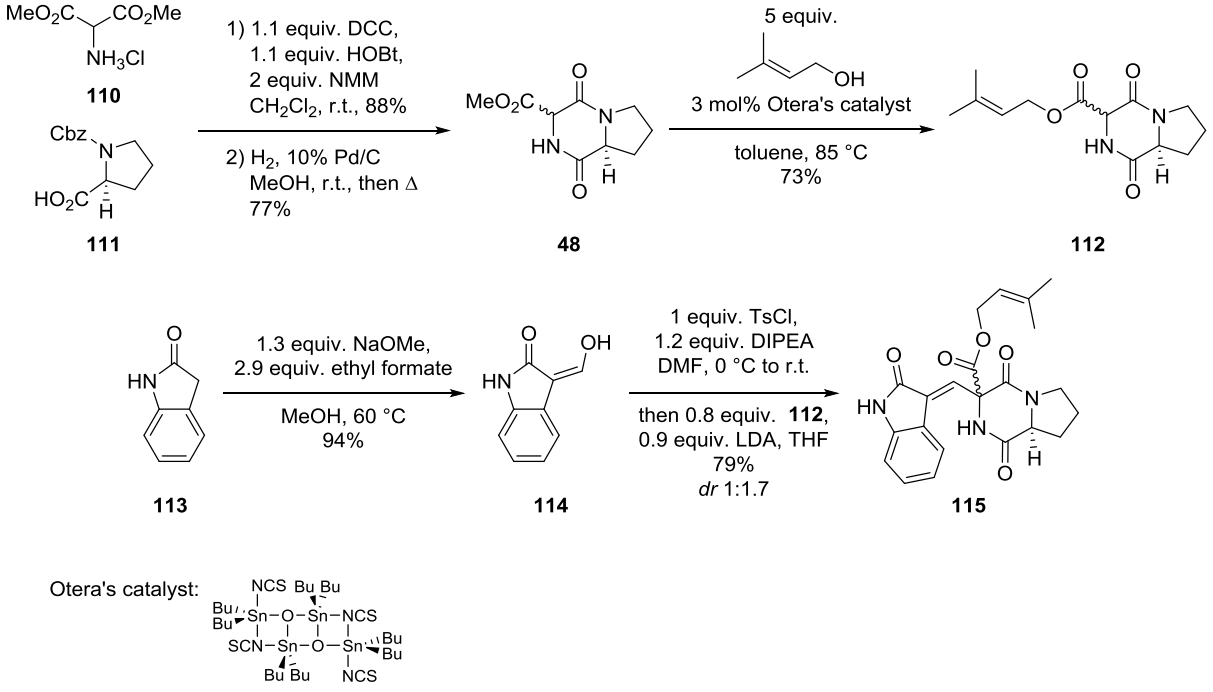


Figure 12: Spirotryprostatin A 86 and B 5.

Different syntheses of spirotryprostatin B **5** have been developed, motivated by its unique structure and limited availability from natural sources (11 mg from a 400 L fermentation).[99-106] The main challenges were the introduction of the spirocyclic center and the prenyl-substituted carbon with the correct stereochemistry. The most recent total synthesis by Trost and Stiles affords the natural isomer of spirotryprostatin B **5** in eight steps.[107]

The diketopiperazine core was constructed from *N*-Cbz-L-proline **111** and dimethyl aminomalonate hydrochloride **110**. Next, the methyl ester in DKP **48** was transesterified with prenyl alcohol in the presence of Otera's catalyst.

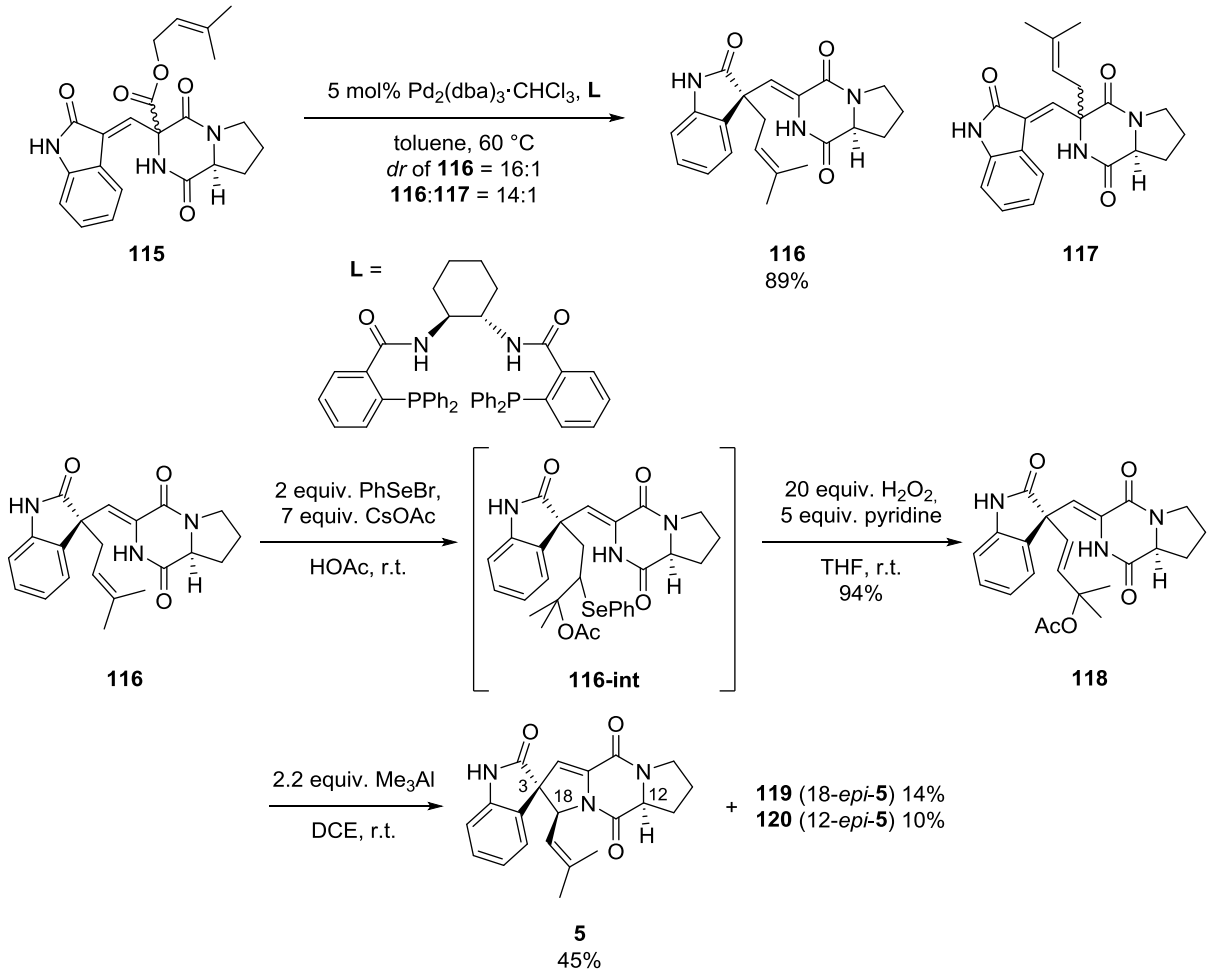
The oxindole group, which was to be introduced on the diketopiperazine ring, was prepared by formylation of **113**. The alcohol **114** was converted to the corresponding vinyl tosylate and the lithium salt of DKP **112** was added. The coupled product **115** was obtained as a 1:1.7 mixture of diastereomers (Scheme 17).



Scheme 17: Synthesis of the key intermediate 115 towards spirotryprostatin B 5 by Trost and Stiles.

The diastereomers **115** were subjected to palladium catalysis to effect the concomitant decarboxylation-prenylation, which yielded a mixture of products **116** and **117**. After a screening of different reaction conditions, the desired product **116** was present as the major compound (Scheme 18).

To accomplish the final cyclization, selenium chemistry was used on **116**. The intermediately obtained selenide **116-int** was oxidized and eliminated to provide the allylic acetate **118**. The final cyclization to **5** was accomplished by generation of an aluminum amide on treatment with trimethylaluminum. Spirotryprostatin B **5** was obtained in 13% overall yield, together with minor amounts of isomers **119** and **120**.



Scheme 18: Total synthesis of spirotryprostatin B 5 *via* a diastereoselective prenylation.

5.3. Cyclotryprostatins

Cyclotryprostatins A **87**, B **88**, C **89** and D **90**, isolated from the secondary metabolites of *Aspergillus fumigatus* BM939, were also identified as inhibitors of the mammalian cell cycle (Figure 13). Compounds **87–90** inhibited the cell cycle progression of tsFT210 cells at the G2/M phase with IC_{50} values of 5.6 µM (**87**), 19.5 µM (**88**), 23.4 µM (**89**) and 25.3 µM (**90**), respectively.

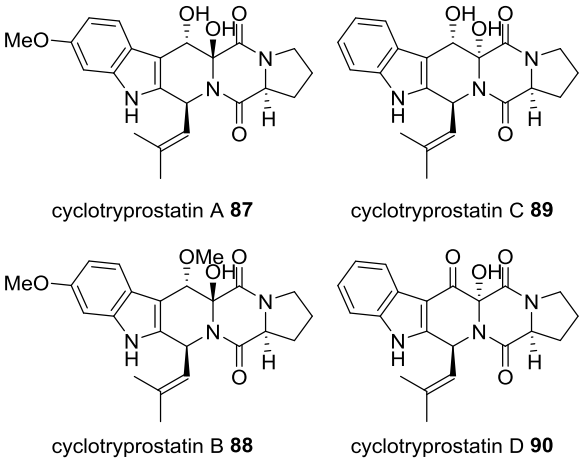
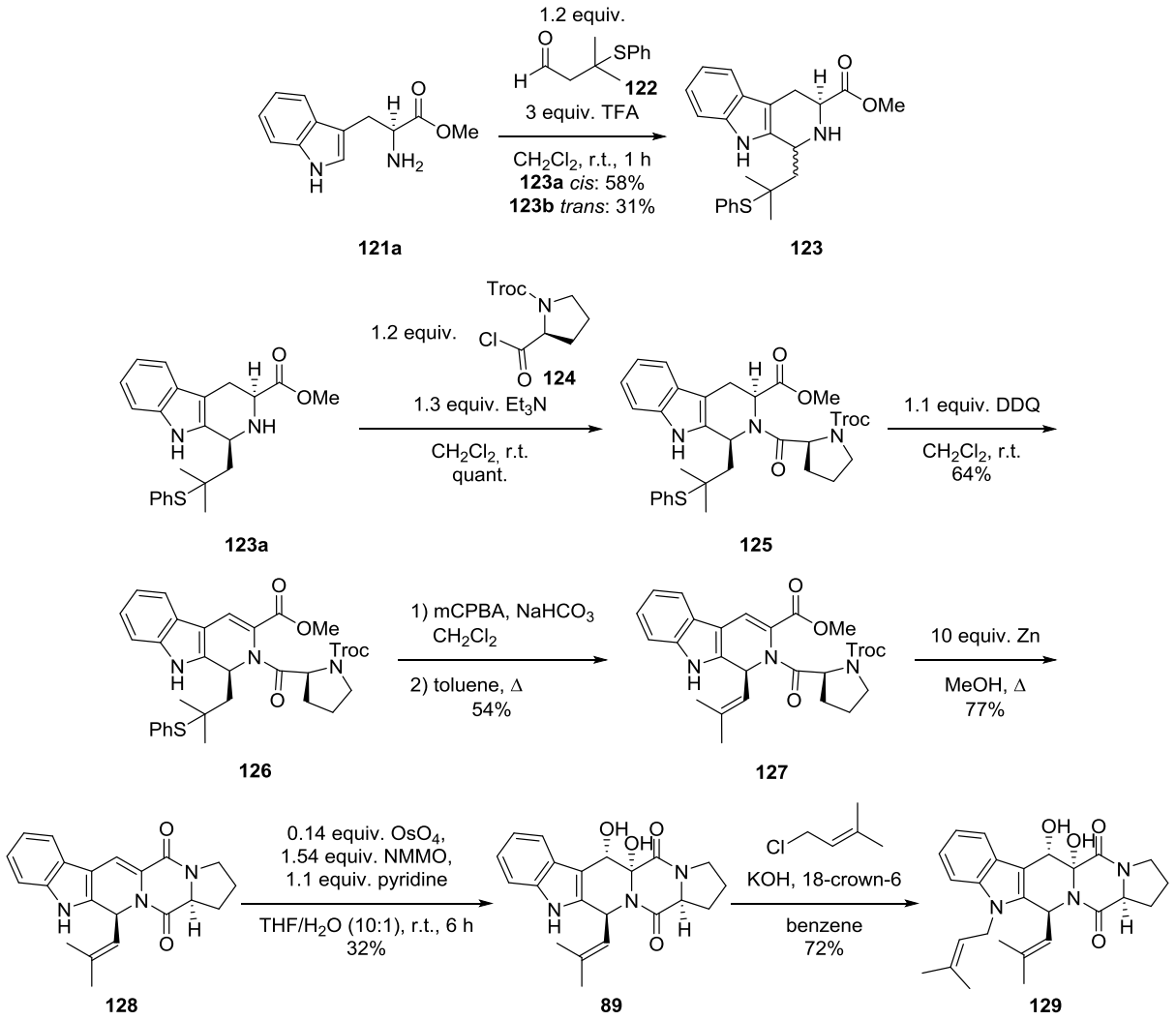


Figure 13: Cyclotryprostatins A-D.

These compounds contain an annulated structure similar to the fumitremorgins. Cyclotryprostatin C **89**, for example, was synthesized as intermediate towards demethoxyfumitremorgin B **129** (Scheme 19).[108] The annulated structure is obtained by a Pictet-Spengler reaction of L-tryptophan methyl ester **121a** with aldehyde **122**. The 1,3-*cis*-β-carboline **123a** was formed as the major product, next to the *trans*-isomer **123b**. Compound **123a** was coupled with *N*-Troc-L-prolyl chloride derivative **124** to afford dipeptide **125**. Next, dehydrogenation with DDQ was performed providing dipeptide analogue **126**. The prenyl group was achieved through dehydrosulfenylation of **126**. Removal of the protecting group of **127** followed by cyclization yielded the pentacyclic compound **128**. Finally, dihydroxylation of the double bond led to cyclotryprostatin C **89**, which was further converted in demethoxyfumitremorgin B **129**.



Scheme 19: Synthesis of cyclotryprostatin C 89 as intermediate towards demethoxyfumitremorgin B 129.

5.4. Fumitremorgins

Fumitremorgin C **7** and demethoxyfumitremorgin C **91** were also isolated from the fermentation broth of *Aspergillus fumigatus* BM939 by Osada *et al.* (Figure 14).[94] Fumitremorgin C **7** was identified as a BCRP-specific inhibitor and thus acts as a chemosensitizing agent, which reverses MDR.[18, 109] However, fumitremorgin C **7** has tremor-inducing activity[110] and causes cell-cycle arrest at the G2/M phase.[15] Therefore, fumitremorgin C analogues are required, which are more selective and display less toxicity.[111-112]

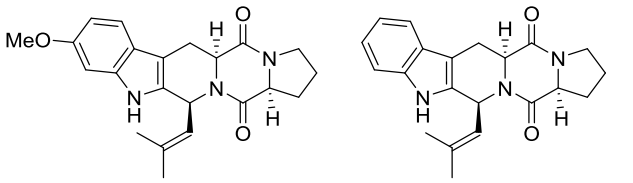
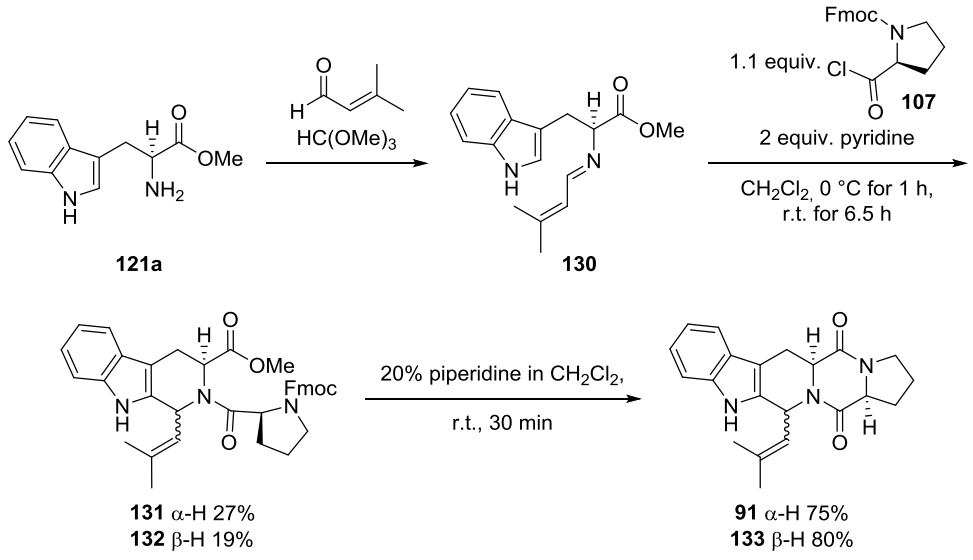


Figure 14: Fumitremorgin C 7 and demethoxyfumitremorgin C 91.

Demethoxyfumitremorgin C **91** is the most effective inhibitor of the mammalian cell cycle of these natural diketopiperazines (Table 2). Removal of the methoxy group on the aromatic ring causes an increase in inhibitory activity with respect to **7**, which is also observed for tryprostatin A **6** and B **85**, and spirotryprostatin A **86** and B **5**, respectively. The minimal inhibitory concentration (MIC) of 0.45 µM for **91** is a tenfold lower than for fumitremorgin C **7** (MIC 4.1 µM).

A concise three-step synthesis of demethoxyfumitremorgin C **91** was reported, which starts off by treating L-tryptophan methyl ester **121a** with 3-methyl-2-butenal in pure trimethyl orthoformate to generate the imine **130** (Scheme 20).[113] The imine was acylated with *N*-Fmoc-L-prolyl choride **107** to afford a 1.4:1 diastereomeric mixture of the annulated dipeptides **131** and **132** *via* an acyliminium Pictet-Spengler condensation. After separation, the diastereomers were subjected to deprotection and simultaneous cyclization in the presence of piperidine to afford the natural product **91** and its diastereomer **133**.



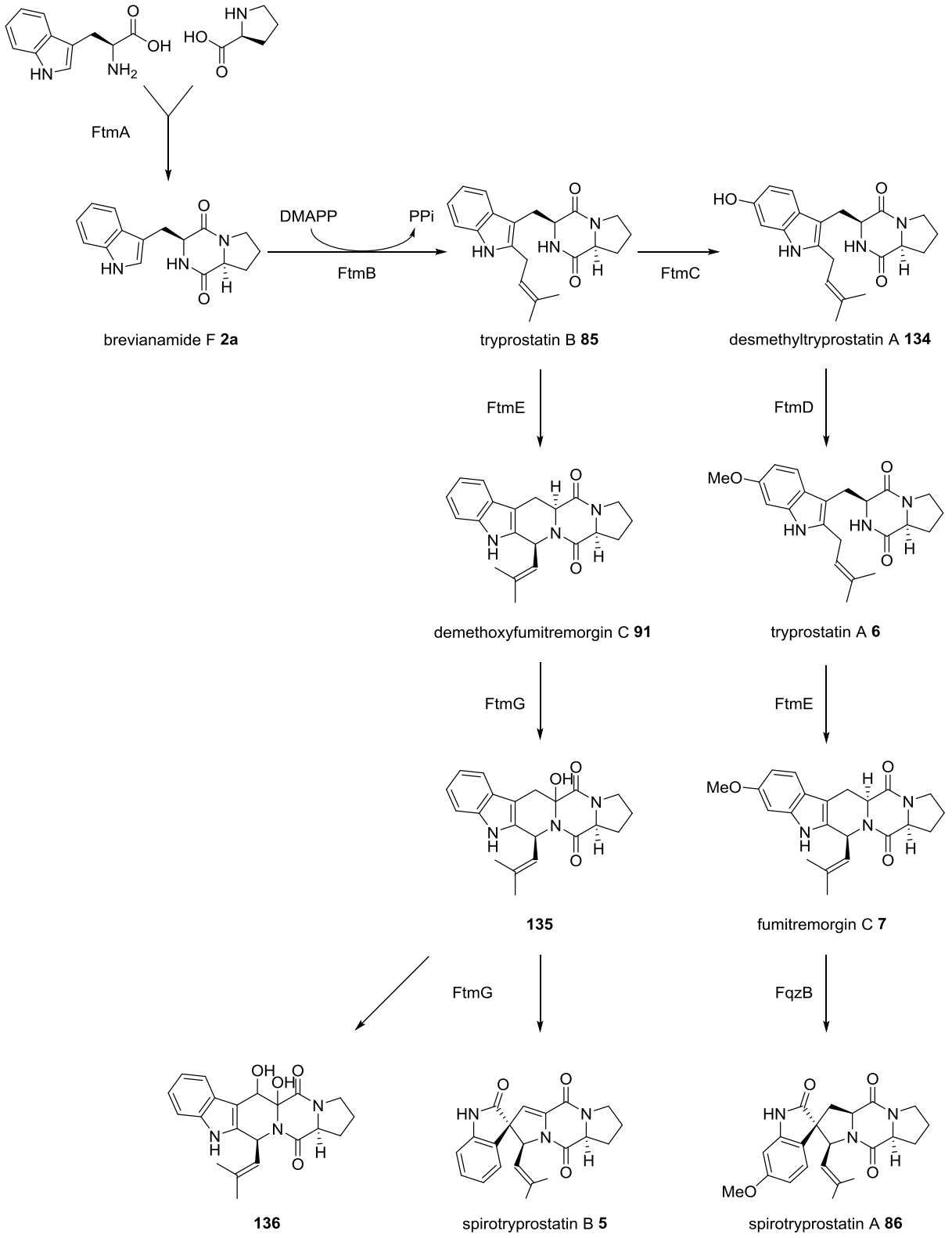
Scheme 20: Synthesis of demethoxyfumitremorgin C 91.

5.5. Biosynthetic pathway

The fumitremorgin biosynthetic pathway is responsible for the biosynthesis of the tryprostatins. Tryprostatins A **6** and B **85** are the biosynthetic intermediates towards fumitremorgin-type alkaloids and are the presumed precursors of the spirotryprostatins (Scheme 21).[114]

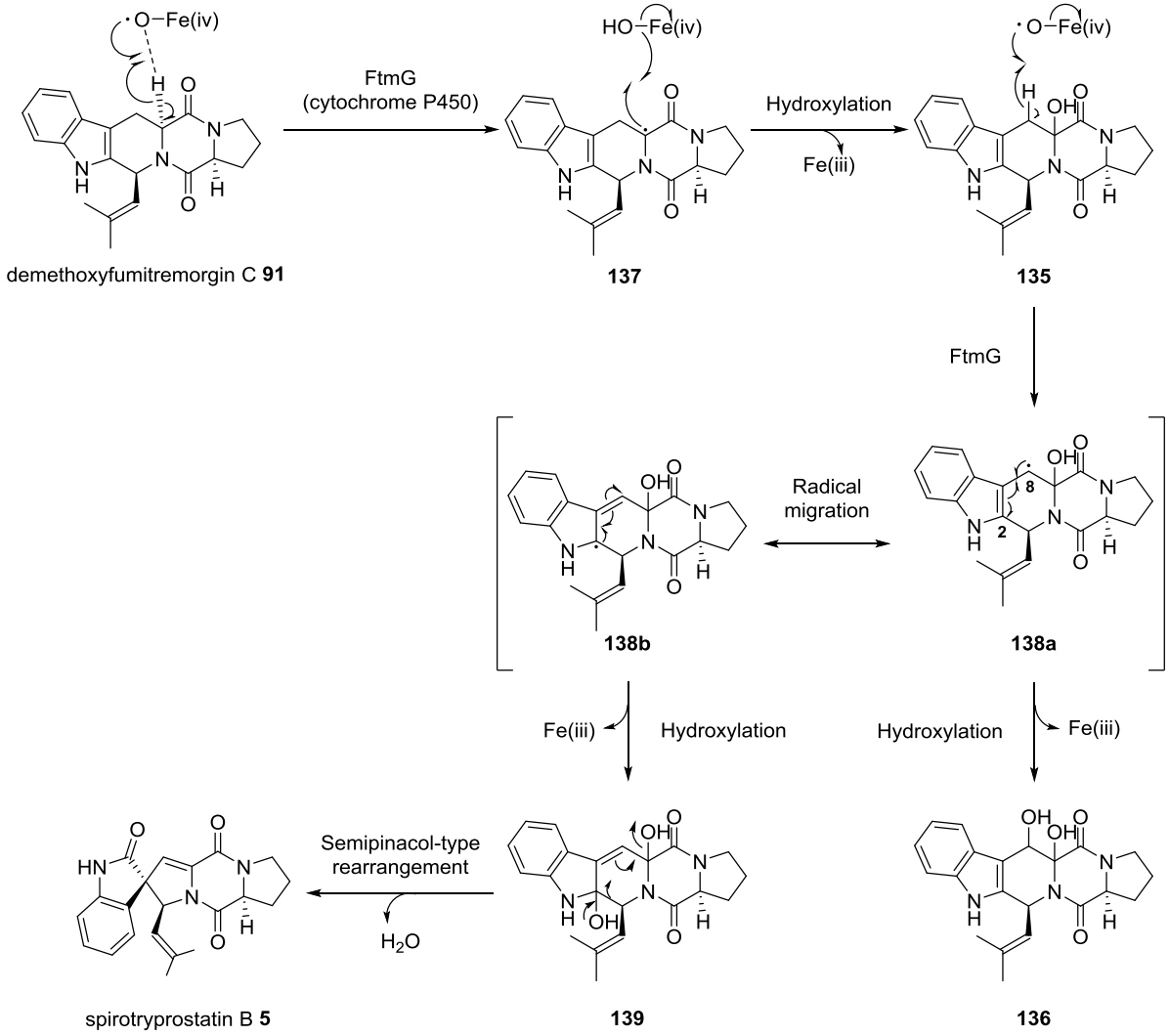
The peptide synthetase FtmA is responsible for the formation of brevianamide F **2a** from L-tryptophan and L-proline.[70] Next, the brevianamide F scaffold is decorated with a prenyl group by prenyltransferase FtmB, which leads to tryprostatin B **85**.[115] FtmC effectuates aromatic hydroxylation of **85** yielding desmethyltryprostatin A **134**,[116] which is subsequently methylated by FtmD resulting in tryprostatin A **6**.[117] When tryprostatin A **6** is exposed to FtmE, the indole ring is fused to the diketopiperazine core to form the pentacyclic fumitremorgin C **7**.[116] The mechanism for spiro-carbon formation, which is involved in the conversion of fumitremorgin C **7** into spirotryprostatin A **86**, is supposedly mediated by FqzB.[118] FqzB is an FAD-containing monooxidase originating from an unrelated gene cluster. This crossover of biosynthetic pathways allows organisms to widen the range of metabolites that they can synthesize. The FqzB enzyme catalyzes a stereoselective epoxidation of the indole double bond between C-2 and C-3 in **7**, which is followed by a semipinacol-type rearrangement resulting in spirotryprostatin A **86**.

Tryprostatin B **85** is also an intermediate in the biosynthesis of spirotryprostatin B **5**. FtmE converts spirotryprostatin B **85** in demethoxyfumitremorgin C **91** through fusion of the indole ring and diketopiperazine moiety. FtmG and not FqzB is responsible for the formation of the spirocentre in **5** from **91**. Exposure of **91** to FtmG does not only afford spirotryprostatin B **5**, but also compounds **135** and **136**, the mono- and dihydroxylated form of **91**, respectively. Structure **136** resembles cyclotryprostatin C **89**, however, the stereochemistry was not determined.



Scheme 21: Proposed biosynthesis of tryprostatins, fumitremorgins and spirotryprostatins in *A. fumigatus* BM939.

The proposed reaction mechanism for the working of enzyme FtmG involves a P450 heme-catalyzed initial radical formation (**137**) and subsequent hydroxylation of **91** (Scheme 22). Upon the second radical formation (**138a**), the radical can migrate to C-2 (**138b**). Hydroxylation of **138b** results in compound **139**, which undergoes a semipinacol-type rearrangement and dehydratation, with concomitant spiro-ring formation, leading to spirotryprostatin B **5**. When the radical in **138a** does not migrate, the subsequent hydroxylation will result in diol **136**.



Scheme 22: Proposed mechanism for spirocarbon formation in 5 and diol 136 *via* a radical route catalyzed by FtmG.

Thus, spirotryprostatins A and B are the result of two different spiro-carbon formation mechanisms. On the one hand, the epoxide route catalyzed by FqzB, furnishes spirotryprostatin A **86**, on the other hand a radical route catalyzed by FtmG, yields spirotryprostatin B **5**.

6. Conclusion

The interest in the diketopiperazine classes discussed above stems from their pharmaceutical importance and diverse, sophisticated structure.

Many of these natural products display important biological activities (mitotic or BCRP inhibition). Fumitremorgin C **7** is a strong BCRP inhibitor, which reverses multidrug resistance of cancer cells. Some compounds display a new mode of action. Tryprostatin A **6**, for example, inhibits microtubules formation by interfering with MAP instead of interacting with tubulin itself. Unfortunately, these compounds also cause negative side effects such as tremor-induction. Analogues are being investigated to find compounds with improved selectivity.[112, 119-122]

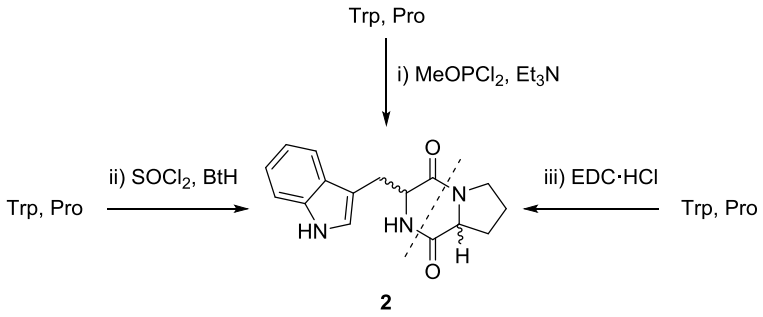
Moreover, synthetic chemists have been attracted by the structural complexity of certain fungal metabolites such as the spirotryprostatins, and as a result these compounds were often chosen as the targets of total syntheses.[118]

Despite the apparent common biosynthetic origin of these natural products, i.e. brevianamide F **2**, uncertainties about the biological pathways leading to these alkaloids still exist. The mechanisms for the intramolecular Diels-Alder reaction remain to be unraveled. The study of the spiro-carbon formation in the biosynthesis of spirotryprostatins has revealed two different mechanisms for spiro-carbon formation. The investigation of the biosynthesis of these complex fungal metabolites may be aided by the development of structural analogues. Biomimetic total syntheses can provide valuable insights into the biosynthetic origin of the diketopiperazines and may aid in discovering the enzymes which are involved in the generation of these complex structures.[118]

III. Results and discussion

1. Synthesis of the cyclo(Trp, Pro) scaffold

The first objective was to develop an efficient route for the synthesis of the basic skeleton, cyclo(Pro, Trp) **2**, starting from the L- and D-enantiomers of proline and tryptophan. Different synthetic routes to obtain the desired compounds were evaluated (Scheme 23). A key step was the coupling of the amino acids. Other aspects like the choice of the protecting group or the order in which to couple the amino acids was also examined. A first strategy was based on phosphorus-assisted amide formation and microwave heating (i).[123] Secondly, benzotriazole activation was tested which involved the activation of the carboxylic group of the amino acids as acylbenzotriazole to perform the coupling (ii).[124] An alternative strategy commonly used in peptide chemistry comprised 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) as a coupling reagent (iii).[125]



Scheme 23: Evaluated syntheses of cyclo(Trp, Pro) 2.

In het course of this research many practical issues arose related to the foaming tendency and the poor solubility of the compounds. The poor solubility in combination with the polarity of certain reported compounds caused many difficulties during purification. The solubility issue is mentioned in the recent review by Sano and Nakao: “In most situations, 2,5-DKP is insoluble in reaction solvents and precipitates as it is formed,” and “In general, intermolecular hydrogen bond formation between the 2,5-DKP rings (C=O···H-N) is responsible for the low solubility of 2,5-DKP in water and various organic solvents.”[126] This was also mentioned by Palacin *et al.*: “In general, DKPs have a low solubility and dissolution required heating and large volumes of solvent.”[127] It was often observed that the DKP (or a derivative) after being formed, did not (completely) redissolve again after the solvent had been evaporated *in vacuo*. Even when the compound was synthesized in a certain solvent (e.g. CH_2Cl_2) and remained in solution during the reaction, attempts to redissolve the compound again after workup in the same solvent often failed. This behavior sometimes complicated purification. While an eluent mixture of a dissolved (and thus diluted) sample was deemed appropriate by TLC, the application of the same eluent mixture during column chromatography or

pTLC could cause formation of an insoluble layer. Subsequent use of a stronger eluent mixture would then result in co-elution of the different products.

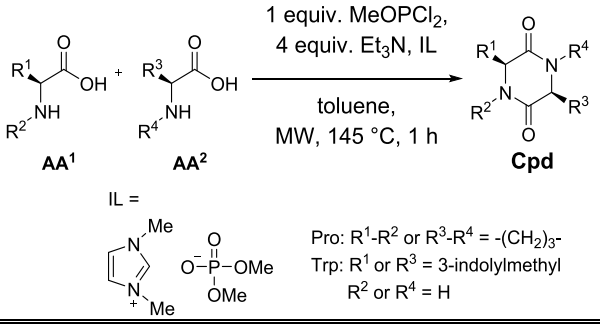
The analysis of crude mixtures mostly had to rely on HPLC-MS analysis since it was difficult to formulate conclusions based on ^{1}H NMR data of the crude and purifications were cumbersome. In the case of the dipeptides, different rotamers made it difficult to interpret ^{1}H NMR spectra. However, ^{1}H NMR analyses were performed on several dipeptides at elevated temperature (60 °C) in DMSO-d6. These results were reported by Delbeke and were not reproduced in this manuscript.[128]

1.1. Diketopiperazine synthesis *via* methyl dichlorophosphite-assisted coupling

A first strategy that was briefly investigated to obtain the diketopiperazine framework involves a phosphite-promoted single-step condensation of unprotected amino acids that uses microwave irradiation-induced heating according to the procedure of Jainta *et al*.[123] This procedure was appealing since it provides a straightforward route to the diketopiperazine ring system without the necessity of introducing protecting groups, which contributes to the atom economy of the reaction. The synthesis of symmetrical homo- and unsymmetrical heterodiketopiperazines was reported. To obtain the heterodiketopiperazines without the corresponding homodiketopiperazines, a small excess of 1.2 equivalents of one amino acid was reported to be necessary.

These reaction conditions were used to attempt the synthesis of different symmetrical and unsymmetrical diketopiperazines (Table 3). This methodology proved to be solely successful for the synthesis of homocoupled cyclo(Pro, Pro) **140** (entry 1). When performing the reaction with glycine or threonine, the desired homodiketopiperazines could not be detected on HPLC-MS (entries 2 and 3). The reaction of tryptophan or glycine and proline did not provide the desired heterodiketopiperazine (entries 4-6). Instead the symmetrical cyclo(Pro, Pro) **140** was observed in all cases despite the addition of a slight excess of the other amino acid. Indeed, the substrates described by Jainta *et al.*[123] were primarily limited to proline and derivatives thereof. This limited substrate scope can explain why the procedure failed with the amino acids used in the present work. No conclusion can be formed about the fate of the starting materials (degradation or preservation) as these were not detected in the HPLC-MS analyses of the resulting crude mixtures.

Table 3: Synthesis of DKPs based on $MeOPCl_2$-assisted coupling.



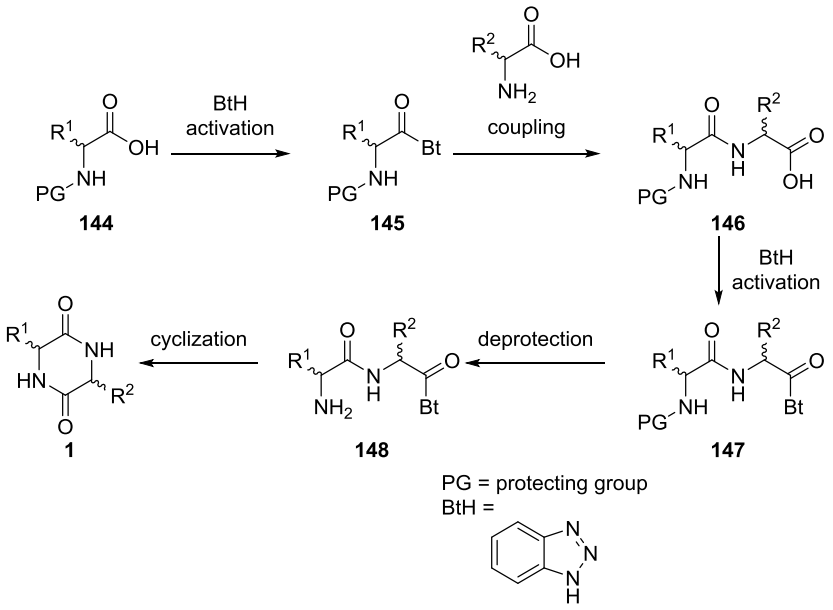
Entry	Cpd		AA^1		AA^2	Yield (%)
1	**140**	1 equiv.	Pro	1 equiv.	Pro	51
2	**141**	1 equiv.	Gly	1 equiv.	Gly	-
3	**142**	1 equiv.	Thr	1 equiv.	Thr	-
4	**2a**	1 equiv.	Trp	1.2 equiv.	Pro	-[a]
5	**2a**	1.2 equiv.	Trp	1 equiv.	Pro	-[a]
6	**143**	1.2 equiv.	Gly	1 equiv.	Pro	-[a]

[a] Only the formation of cyclo(Pro, Pro) **140** was detected on HPLC-MS.

1.2. Diketopiperazine synthesis *via* benzotriazole-assisted coupling

The 1*H*-benzotriazole carboxylic acid activation methodology is well-known and was extensively investigated by Katritzky and coworkers.[129-133] The activation of amino acids with benzotriazole requires only the protection of the amino group and tolerates other functional groups.[134] Moreover, N-acylbenzotriazoles are stable crystalline compounds, which makes them easy to handle. Benzotriazole has been used as an efficient coupling reagent in the synthesis of peptides and derivatives thereof and was investigated in an alternative synthetic route towards the diketopiperazine scaffold (Scheme 24).[124]

The benzotriazole-assisted approach was chosen because of the previous experience with this methodology at our department (SynBioC, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University). Bt-activated amino acids were efficiently synthesized and coupled with heterocyclic amines and amino acids using microreactor technology.[135-136] Moreover, this methodology has already been used for the synthesis of 2,5-diketopiperazines.[137] The planned synthesis starts with the N-protected amino acid **144**, which undergoes activation of its carboxyl group *via* introduction of benzotriazole (**145**). The activated amino acid **145** is subsequently coupled to a second, unprotected amino acid. The resulting dipeptide **146** is again activated by the introduction of benzotriazole at the C-terminus (**147**). Deprotection of the activated dipeptidoyl benzotriazole **147** should lead to **148**, which eventually should result in the desired diketopiperazine **1**.

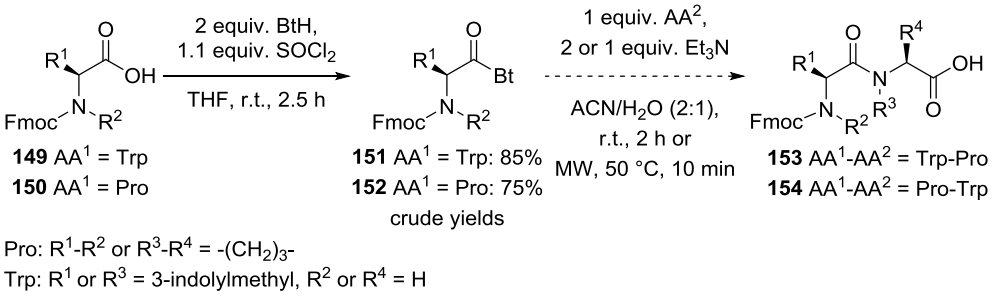


Scheme 24: General synthetic route based on the benzotriazole methodology.

Two protecting groups were evaluated for this synthesis, namely the fluorenylmethylcarbonyl- (Fmoc-)group and the benzyloxycarbonyl- (Cbz-)group. The frequently used *tert*-butyloxycarbonyl- (Boc-)group was not evaluated due to the incompatibility of the benzotriazole-activated dipeptide with acidic conditions typically used for deprotection.

1.2.1. Fmoc protection

The synthesis starts with the *in situ* formation of BtS(O)Bt from thionyl chloride and benzotriazole.[133] Subsequently, the Fmoc-protected amino acids tryptophan **149** or proline **150** were added to yield the activated amino acids **151** and **152**, respectively. Both compounds were obtained in good crude yield (Scheme 25).



Scheme 25: Benzotriazole methodology using Fmoc-protected amino acids.

In the next step, the activated amino acids **151** and **152** were coupled with the unprotected amino acids proline and tryptophan, respectively. The reactions were performed at room temperature and under microwave heating. In both cases the recovery of the desired dipeptide (**153** or **154**) after workup was very low. The poor recovery of dipeptides **153** and **154** in this reaction step can be

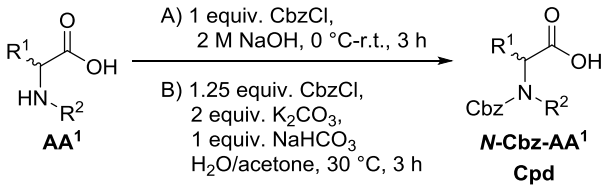
attributed to the use of base, which can cause deprotection of the Fmoc group resulting in side reactions.[138] Therefore, the use of Fmoc as protecting group was abandoned.

1.2.2. Cbz protection

Alternatively, the Cbz protecting group was used, which can be removed *via* hydrogenolysis and is stable in the presence of base. Two methods were compared to achieve the introduction of the protecting group on the unprotected amino acids. The first required the simultaneous addition of CbzCl and a sodium hydroxide solution to keep the pH stable,[139] which was not very convenient (Table 4, reaction conditions A). For the second method the amino acid was dissolved in an aqueous solution of $NaHCO_3$ and K_2CO_3, and acetone (Table 4, reaction conditions B).[140] Both procedures provided the desired compounds in pure form and with good yields (Table 4). The latter procedure was more practical. Moreover, the first procedure describes precipitation of the desired compounds upon acidification of the aqueous solution. However, in the case of the proline derivatives **111** and **156** a viscous oil was obtained, which could not be filtered off.

The chosen procedure B provided Cbz-Trp **155a** or **155b** as a strong foaming oil upon evaporation of the organic solvent after extractive workup, which made it difficult to remove all the solvent. Almost all products, which were synthesized during this PhD thesis were obtained as foams. In this case, co-evaporation of residual volatiles from dichloromethane gave the amino acids **155a** and **155b** as white-grey powders making them easier to handle.

Table 4: Cbz-protection of amino acids.



Pro: R^1-R^2 = -$(CH_2)_3$-

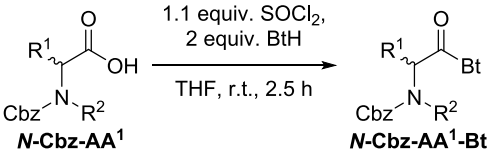
Trp: R^1 = 3-indolylmethyl, R^2 = H

Entry	Cpd	AA[1]	Reaction conditions	Yield (%)	Physical state
1	**155a**	L-Trp	A	90	Powder
2	**155a**	L-Trp	B	92	Powder
3	**155b**	D-Trp	B	94	Powder
4	**111**	L-Pro	A	97	Oil
5	**111**	L-Pro	B	98	Oil
6	**156**	L-Hyp	B	88	Oil

1.2.3. Benzotriazole-activation of N-protected amino acids

Benzotriazole activation of the Cbz-protected amino acids was achieved using a procedure described by Katritzky *et al*. (Table 5).[133] The reaction provided the activated amino acids **157** and **158** in good crude yields. Occasionally, some traces of starting material could be found. The main impurity in the crude product consisted of some remaining benzotriazole. The presence of benzotriazole is no problem, since it will be released in the next step anyhow and added in excess in the second benzotriazole activation step. The Bt-activation of *N*-Cbz-hydroxyproline (Hyp) **156** failed to provide the desired compound **159** as the sole product and resulted in the formation of several products. HPLC-MS showed several more polar side products with higher mass than the expected compound. These may be the result of self condensation, by the reaction of **156** with another molecule of already activated **159**, due to the presence of the unprotected hydroxyl group.

Table 5: Benzotriazole activation of Cbz-protected amino acids.



***N*-Cbz-AA^1** ***N*-Cbz-AA^1-Bt**

Cpd

Pro: R^1-R^2 = -$(CH_2)_3$-

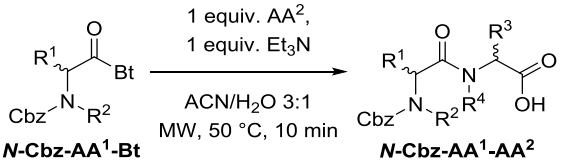
Trp: R^1 = 3-indolylmethyl, R^2 = H

Cpd	AA^1	Crude yield (%)
157a	L-Trp	70
157b	D-Trp	94
158	L-Pro	91
159	L-Hyp	Complex reaction mixture

1.2.4. Dipeptide synthesis with benzotriazole-activated amino acids

The condensation of the benzotriazole-activated monomers with an unprotected amino acid was accomplished according to a procedure from Katritzky *et al.* yielding the dipeptides **160** to **162**.[133] The coupling of both amino acids was executed under microwave heating so a short reaction time of ten minutes sufficed. The crude products (**160**-**162**) were used as such in the second benzotriazole activation step.

Table 6: Condensation to the dipeptide.



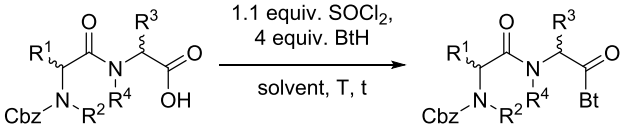
Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Cpd	AA^1	AA^2	Crude yield (%)
160	L-Trp	L-Pro	72
161	D-Trp	L-Pro	92
162	L-Pro	D-Trp	93

1.2.5. Benzotriazole-activation of N-protected dipeptides

In the next step the dipeptides were again activated *via* the introduction of a benzotriazole group at the C-terminus of the dipeptide with procedures from the Katritzky research group (Table 7).[130, 137] These procedures started with the *in situ* formation of BtS(O)Bt. A large excess of benzotriazole was used. When the reaction was started at lower temperature (-10 °C) (entry 3 versus entry 4),[137] the crude yield was slightly better. The activated dipeptides **163** and **164** were obtained in acceptable purity (more than 95% pure based on HPLC analysis) so no further purification was performed.

Table 7: Benzotriazole activation of dipeptides.



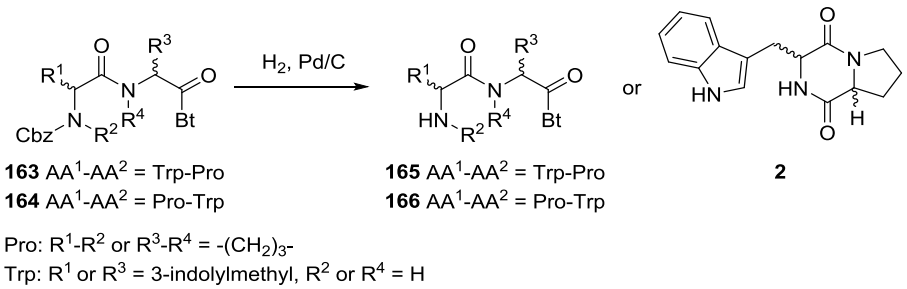
Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Entry	Cpd	AA^1	AA^2	Solvent	T, t	Crude yield (%)
1	**163a**	L-Trp	L-Pro	CH_2Cl_2	-10 °C, 1 h Then r.t., 1 h	58
2	**163b**	D-Trp	L-Pro	CH_2Cl_2	-10 °C, 1 h then r.t., 1 h	87
3	**164**	L-Pro	D-Trp	CH_2Cl_2	-10 °C, 1 h then r.t., 1 h	78
4	**164**	L-Pro	D-Trp	THF	0 °C, 2 h	68

1.2.6. Hydrogenolysis of N-Cbz-protected dipeptides

Subsequently, the removal of the N-protecting group, whether or not followed by spontaneous ring closure, was aspired in accordance to the procedure described by Monbaliu *et al*. (Scheme 26).[137] The synthesis of the *trans*-diketopiperazines containing one unnatural amino acid was examined first because of the anticipated easier formation of the thermodynamically more stable *trans*-DKP. Dipeptide **164** was foremost investigated as this allows the introduction of the more expensive D-Trp later on in the pathway, which avoids losses during the first protection or activation steps. While the unnatural amino acid, D-Pro, could be introduced by the *trans*-selective cyclization/epimerization method of Monbaliu *et al.* (*vide infra*).[137]

However, hydrogenolysis in ethanol did not lead to the desired cyclized product **2**, nor did it provide the N-deprotected benzotriazole-activated dipeptide **165** or **166**. Instead, the dipeptide esters **167** and **168** were present as the main products, in which the benzotriazolyl moiety was replaced with an ethoxy group and no deprotection had taken place (Table 8, entries 1-2). The benzotriazole-activated carboxyl group reacts with the alcoholic solvent used, leading to the ester or with traces of water leading to the acid (**162**, entry 3). However, this was not a problem during the hydrogenolysis and cyclization of the dipeptides studied by Montbaliu *et al.*[137] No traces of deprotection were detected leading to the supposition that the palladium catalyst was inactivated by the nitrogen atoms present in benzotriazole, the indole or the amide bond. A larger amount of palladium catalyst was added (entry 4), but still no deprotection was detected. The reactions were also performed in the presence of a catalytic amount of acid without success (entries 5[141]-6). Since the reaction of the dipeptide with the alcoholic solvent proceeded so easily, EtOAc and CH_2Cl_2 were evaluated as solvent (entries 7-8[142]). In these instances, the starting material **164** was recovered.



Scheme 26: Desired products after removal of the Cbz protecting group from dipeptides 163-164.

Cyclohexene and ammonium formate were evaluated as alternative sources of hydrogen (entries 9[143]-10). The use of ammonium formate was promising and led to the deprotected dipeptide **169b**, which had also undergone reaction with the solvent methanol. The reaction with ammonium formate was repeated with several other non-alcoholic solvents (entries 11-14). No

desired product was formed and side reactions had taken place resulting in complex reaction mixtures of polar compounds. One of these side products was tentatively attributed as the dipeptide amide Trp-Pro-$CONH_2$ resulting from the reaction of **164** with the ammonia released during decomposition of ammonium formate.

As another catalyst, $PdCl_2$, was used, which led to the removal of the Cbz group (entry 15) and concomitant esterification by the alcoholic solvent, yielding **169b**. Switching the solvent to dichloromethane, to prevent esterification, led to precipitation of the product upon addition of hydrochloric acid, whereupon no reaction occurred (entry 16).

Hydrogenolysis is the standard method for deprotection of the Cbz group.[144] There was no obvious reason why the hydrogenolysis should not work as this had been reported by Monbaliu *et al.* for related dipeptides. Therefore, only hydrogenolysis involving Pd catalysts were tested.

Moreover, in view of the tentative idea of translating the DKP **2** synthesis into a continuous flow process, the removal of the Cbz group through hydrogenolysis was envisaged. Hydrogenolysis can be transferred into a flow process using specialized equipment e.g. the H-Cube Mini reactor, available at the department. This offers the possibility of pumping a dipeptide solution through a fixed bed of Pd/C catalyst, allowing the easy recuperation and reuse of this relatively expensive catalyst, while generating the explosive H_2 *in situ* in small quantities. Unfortunately, the synthesis of DKP **2** from dipeptides **164** *via* hydrogenolysis did not look very promising.

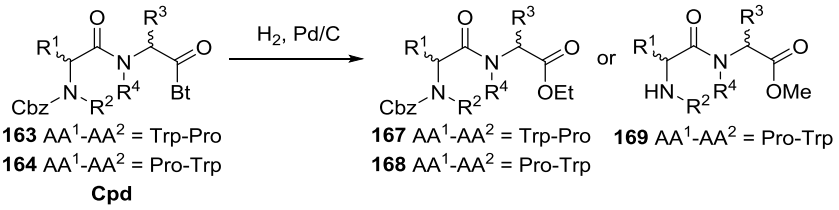
Acidic (e.g. HBr) or alkaline (e.g. KOH in MeOH) deprotection strategies were not tested as these could induce unwanted epimerization. However, other mild methods for Cbz removal (e.g. $n$$Bu_4NF$) can be suggested for further research.[145]

In both cases where deprotection was successful (entries 10 and 15), an alcoholic solvent was used leading to esterification. The alkoxy group is a good leaving group permitting cyclization, so the desired DKP could be obtained from **169** under the right conditions. However, this would abolish the mere reason to use the benzotriazole-based synthetic route.

During concomitant research, the synthesis of the benzotriazole activated L-tryptophan **157** using microreactor technology has been developed at our department as part of another PhD thesis.[136] The amino acid **157** was subsequently coupled in flow with L-ProOMe·HCl **183a** providing the dipeptide *N*-Cbz-L-Trp-L-ProOMe **181a**. Unfortunately, substantial epimerization had occurred (6:1 *cis*:*trans*) as a result of the elevated temperature (130 °C) required to obtain full conversion in the limited residence time (= reaction time) of 30 min in the reactor.

The dipeptide methyl ester can be formed without epimerization and in only one step with carbodiimide mediated coupling (*vide infra*). As a result, further steps towards the deprotection of **163** or **164** were not investigated within the timeframe of this work.

Table 8: N-deprotection of the dipeptides 163-164.



163 AA^1-AA^2 = Trp-Pro
164 AA^1-AA^2 = Pro-Trp
Cpd

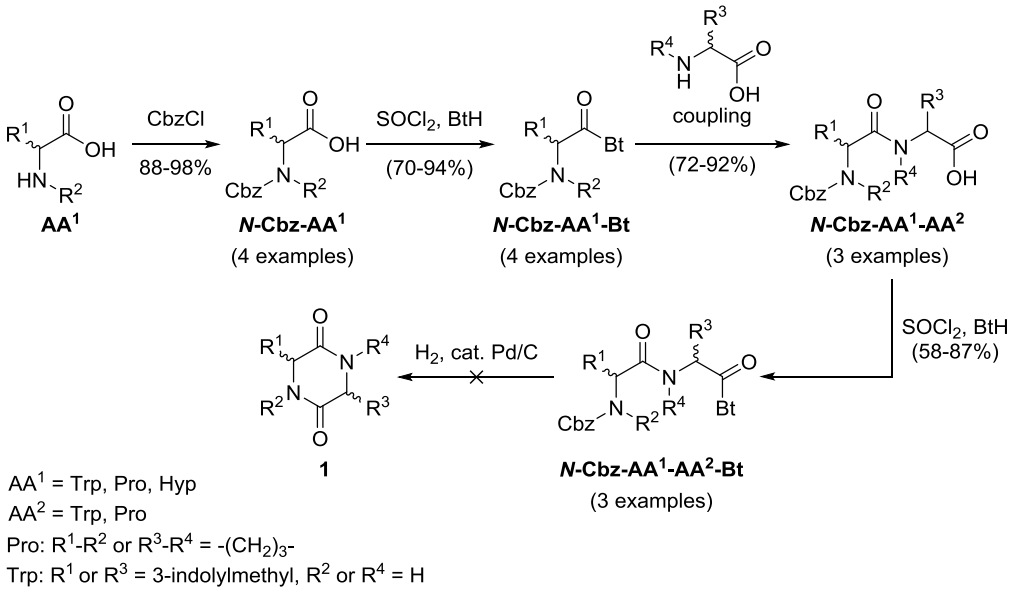
167 AA^1-AA^2 = Trp-Pro
168 AA^1-AA^2 = Pro-Trp

169 AA^1-AA^2 = Pro-Trp

Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Entry	**AA^1**	**AA^2**	**Reaction conditions**	**Result**
1	L-Trp	L-Pro	5 bar H_2, 10 wt% Pd/C EtOH, r.t., 18 h	**163a/167**
2	L-Pro	D-Trp	5 bar H_2, 10 wt% Pd/C EtOH, r.t., 18 h	**164/168**
3	L-Pro	D-Trp	5 bar H_2, 10 wt% Pd/C EtOH, r.t., 23 h	**164/168/162**
4	L-Pro	D-Trp	5 bar H_2, 100 wt% Pd/C EtOH, r.t., 20 h	**168**
5	L-Pro	D-Trp	5 bar H_2, 10 wt% Pd/C, cat. HOAc EtOH, r.t., 20 h	**164/168**
6	L-Pro	D-Trp	5 bar H_2, 10 wt% Pd/C, cat. HCl EtOH, r.t., 1 d	**168**
7	L-Pro	D-Trp	5 bar H_2, 50 wt% Pd/C EtOAc, r.t., 65 h	**164**
8	L-Pro	D-Trp	5 bar H_2, 50 wt% Pd/C, 1 equiv. DIPEA CH_2Cl_2, r.t., 1 d	**164**
9	L-Pro	D-Trp	Cyclohexene, 50 wt% Pd/C EtOH, Δ, 6 h	**164/168**
10	L-Pro	D-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C MeOH, Δ, 2 h	**169b**
11	L-Pro	D-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C EtOAc, Δ, 3 h	Complex reaction mixture
12	L-Pro	D-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C CH_2Cl_2, Δ, 19 h	Complex reaction mixture
13	L-Pro	D-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C THF, Δ, 1 h	Complex reaction mixture
14	L-Pro	D-Trp	4 equiv. $HCOONH_4$, 5 wt% Pd/C *i*PrOH, MW, 83 °C, 10 min	Complex reaction mixture
15	L-Pro	D-Trp	5 bar H_2, 0.3 equiv. $PdCl_2$, 4 equiv. HCl MeOH, r.t., 3 d	**169b**
16	L-Pro	D-Trp	5 bar H_2, 0.3 equiv. $PdCl_2$, 4 equiv. HCl CH_2Cl_2, r.t., 3 d	**164**

A final overview of the benzotriazole synthetic pathway that was examined, is presented in Scheme 27.

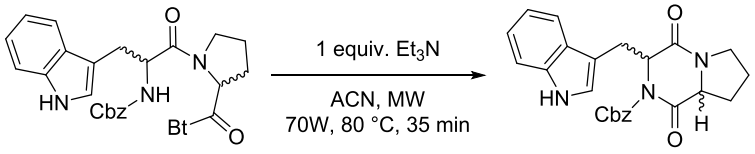


Scheme 27: Overview of the synthetic pathway using the benzotriazole activation methodology starting from Cbz-protected amino acids. Crude yields are depicted between parentheses.

1.2.7. Trans-selective cyclization/epimerization

A tandem *trans*-selective cyclization/epimerization of N-Cbz-dipeptidoyl benzotriazoles has been described by Monbaliu *et al*.[137] The procedure allows ring formation of the benzotriazole-activated dipeptides without removal of the N-protecting group. With the required substrates already in hand, these conditions were applied on the compounds **163a** and **163b** (Table 9). Dipeptide **163a**, made up from two L-amino acids, undergoes epimerization at the proline stereocenter under the current reaction conditions and the *trans*-diketopiperazine **170a** was formed (entry 1). On the other hand, starting from the D,L-dipeptide **163b** the stereochemistry of the amino acids was retained in the cyclized product **170b** (entry 2).

Table 9: Cyclization of Cbz-dipeptidoyl benzotriazoles 163a and 163b.

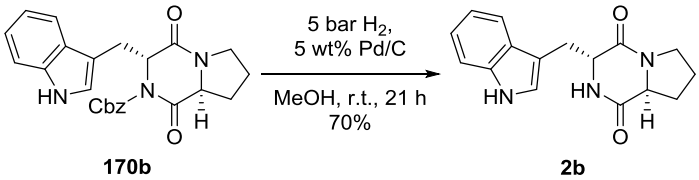


163 170

Entry		AA¹	AA²	Cpd	AA¹	AA²	Yield (%)
1	**163a**	L-Trp	L-Pro	**170a**	L-Trp	D-Pro	96
2	**163b**	D-Trp	L-Pro	**170b**	D-Trp	L-Pro	41[a]

[a] Isolated yield after column chromatography.

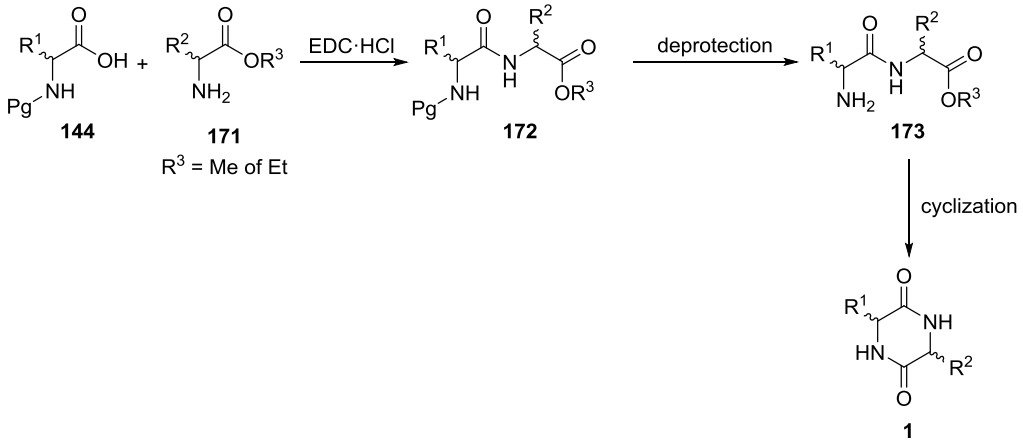
Hydrogenolysis of compound **170b** gave the desired DKP **2b** (Scheme 28). However, this procedure is limited to the synthesis of *trans*-diketopiperazines. Moreover, it takes up to 6 steps to get the desired compounds.



Scheme 28: Hydrogenolysis of Cbz-protected DKP 170b.

1.3. Diketopiperazine synthesis *via* carbodiimide-assisted coupling

A third strategy to obtain the desired diketopiperazines used EDC·HCl as a coupling reagent. Carbodiimides are commonly used coupling reagents in standard peptide chemistry.[146-147] Scheme 29 shows the envisaged synthetic route, which is notably shorter than the one using the benzotriazole methodology. However, the use of appropriate protecting groups is required. In the first step a N-protected amino acid **144** and the ester of a second amino acid **171** are coupled in the presence of EDC. Subsequently, the protecting group is removed from the dipeptide **172**, upon which (spontaneous) cyclization of **173** affords the diketopiperazine **1**.



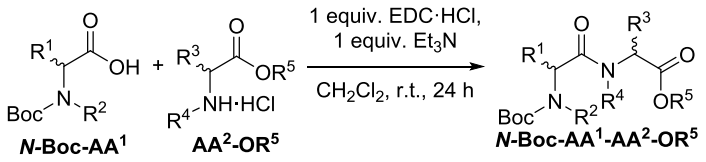
Scheme 29: General synthesis of the DKP scaffold 1 *via* EDC-assisted coupling.

1.3.1. EDC-assisted coupling

In the current synthetic route, several N-Cbz- and N-Boc-protected tryptophan, proline and hydroxyproline amino acids were submitted to EDC-mediated coupling with methyl or ethyl esters of these amino acids. These products were commercially available, only the *N*-Cbz-D-Trp **155b** was synthesized from D-Trp.[125] Evaporation of the organic phase after extractive workup resulted again in strong foaming of the desired compounds **174**-**182**. These compounds were already quite pure

(more than 95% pure based on HPLC analysis) and were used without further purification in the next step (Table 10 and Table 11).

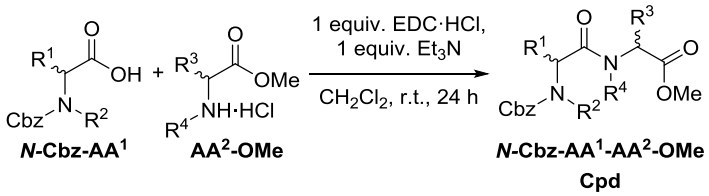
Table 10: EDC-assisted coupling starting from N-Boc-protected amino acids.



Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Cpd	AA^1	AA^2	R^3	Crude yield (%)
174	L-Pro	L-Trp	Me	85
175	L-Hyp	L-Trp	Me	60
176	L-Trp	Gly	Et	73
177	L-Pro	L-Pro	Me	96
178	L-Trp	L-Hyp	Me	67
179	L-Hyp	Gly	Et	67

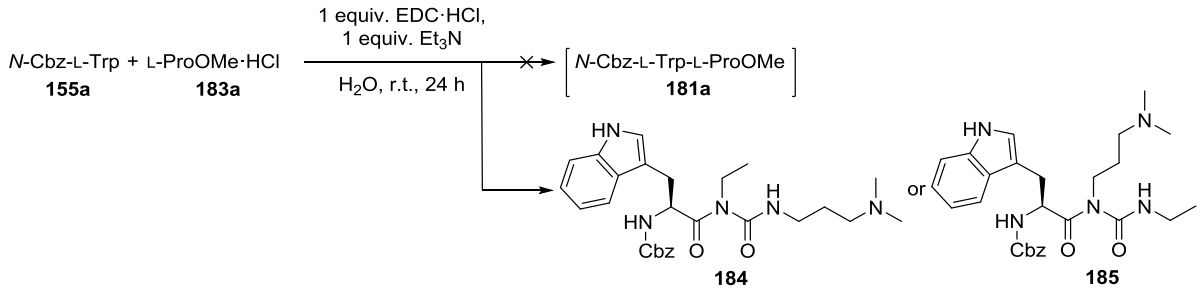
Table 11: EDC-assisted coupling starting from N-Cbz-protected amino acids.



Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Cpd	AA^1	AA^2	Crude yield (%)
180a	L-Pro	L-Trp	95
180b	L-Pro	D-Trp	67
181a	L-Trp	L-Pro	73
181b	L-Trp	D-Pro	76
181c	D-Trp	L-Pro	89
181d	D-Trp	D-Pro	76
182a	L-Trp	L-Hyp	91
182b	D-Trp	L-Hyp	70

The procedure with EDC·HCl was a straightforward method for the coupling of the amino acids. Unfortunately, the coupling reaction was performed in dichloromethane, a halogenated solvent. At SynBioC the use of these solvents is discouraged and avoided if possible to prevent any release into the environment. Firstly, water was used as a benign alternative solvent.[148] However, the starting material **155a** did not dissolve in water. The desired dipeptide **181a** was not detected *via* HPLC-MS, only a side product such as **184** or **185** resulting from O- to N-acyl-transfer during the activation of tryptophan with EDC was formed (Scheme 30).

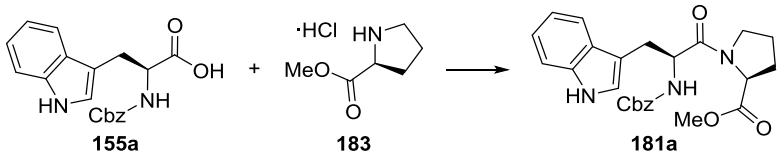


Scheme 30: EDC coupling in water.

Next, methanol was screened as solvent to perform the coupling. The following hydrogenolysis and cyclization steps were also to be performed in methanol (*vide infra:* 1.3.3 and 1.3.4). Hence, if these three steps could be conducted in the same solvent, a one pot procedure might be possible, avoiding several workups.

In the absence of additional base, esterification of *N*-Cbz-L-Trp **155a** took place and only a trace of dipeptide **181a** was present (Table 12, entry 1). The addition of base to the reaction gave more formation of dipeptide, alongside esterification to *N*-Cbz-L-TrpOMe **186** and formation of the side product **184**/**185** resulting from acyltransfer (entry 2). Performing the reaction at lower temperature eliminated the esterification side product **186**, but still a significant amount of **184**/**185** was present (entry 3).

Table 12: EDC-coupling in methanol.

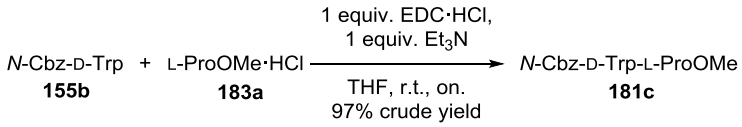


155a **183** **181a**

Entry	Reaction conditions	Result[a]
1	1 equiv. EDC·HCl MeOH, r.t., 24 h	Trace of **181a** *N*-Cbz-L-TrpOMe **186**
2	1 equiv. EDC·HCl, 1 equiv. Et_3N MeOH, r.t., 24 h	**181a**/(**184+185**+**186**) 1/1.25
3	1 equiv. EDC·HCl, 1 equiv. Et_3N MeOH, 0 °C-r.t., 24 h	**181a**/(**184**+**185**) 1/1

[a] Ratio determined on the 280 nm UV-signals in the HPLC chromatogram.

The coupling was also performed in THF. The crude yield was good, but more side products were formed (Scheme 31). After deprotection of the crude dipeptide **181c** through hydrogenolysis (for the conditions *vide infra*) and spontaneous cyclization, cyclo(D-Trp, L-Pro) **2b** could not be isolated by recrystallization. Using column chromatography the pure diketopiperazine **2b** was recovered in low yield (34% over 2 steps). This yield was lower compared to the yield of **2b** (79% over 2 steps, *vide infra*) obtained using dichloromethane as solvent for the amino acid coupling. Hence, this solvent was also abandoned.



Scheme 31: EDC-mediated coupling in THF.

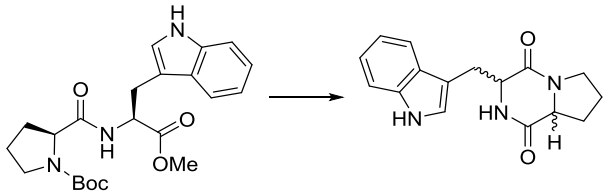
Since the conversion to the desired product remained low in the alternative solvents, dichloromethane remained the solvent of choice for the coupling. However, other solvents are still possible, but were not investigated within the timeframe of this work. The use of other reagents or additives may still allow the amide bond formation in high yield and purity in other solvents than dichloromethane.

1.3.2. Boc deprotection

The Boc protecting group was removed thermally or by means of a strong acid. In the first instance the deprotection of **174** was attempted at elevated temperature using microwave-assisted heating, with the aim of spontaneous cyclization to the DKP ring according to the procedure of Pérez-Picaso *et al.* (Table 13, entry 1).[149] The diketopiperazine **2** was isolated as a mixture of diastereomers. The diastereomeric ratio was 1:1.4 (*cis*:*trans*). The absolute stereochemistry was not determined.

To avoid the undesired epimerization, the temperature was lowered to 150 °C. In compensation, the reaction time was augmented to 15 minutes. A mixture of *cis*- and *trans*-diastereomers **2** was still detected (entry 2). An alternative procedure involved deprotection in the microwave reactor in the presence of acetic acid, which again resulted in a mixture of diastereomers **2** (entry 3).[150] Next, the solvent was switched to aprotic solvents like acetonitrile (ACN) and dimethyl sulfoxide (DMSO). Indeed, a protic solvent like water can promote enolization of the carbonyl function, leading to epimerization and producing the thermodynamically more stable *trans*-configuration. The temperature was also lowered further to 130 °C. Only a trace of the desired DKP **2** was detected after reaction in ACN, and the starting material **174** was largely retrieved (entry 4). This reaction could not be reproduced. The DKP **2** was not detected again, even after prolonged reaction times or augmented temperature (entries 5, 6). In DMSO the DKP **2** was also detected after 15 minutes, besides starting material **174**, unprotected dipeptide **169** and some unidentified side products (entry 7). After prolonged heating all the starting material **174** was converted to degradation products (entry 8). A final attempt for thermal removal of the Boc moiety was based on refluxing the dipeptide in *N*-methyl-2-pyrrolidinone (NMP). No conversion of the starting material **174** took place (entry 9).[151]

Table 13: Boc deprotection of 174 and cyclization under microwave heating.



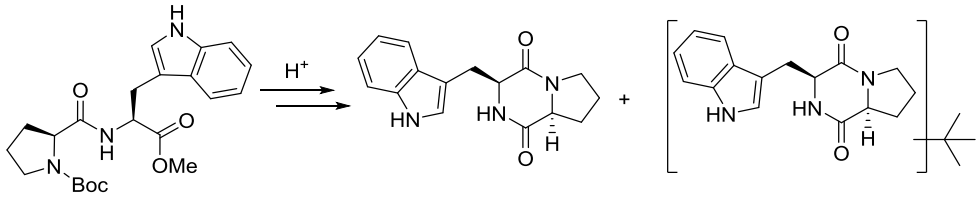
174 **2**

Entry	Reaction conditions	Crude yield 2 (%)
1	H_2O MW, 250 °C, 10 min	97 *dr* = 1:1.4 *cis*:*trans*[a] *cis*: 38%[b] *trans*: 38%[b]
2	H_2O MW, 150 °C, 15 min	87 *dr* = 1:1.5 *cis*:*trans*[c]
3	HOAc/H_2O (1:1) MW, 160 °C, 5 min	*dr* = 1:1.5 *cis*:*trans*[c]
4	ACN MW, 130 °C, 15 min	Trace
5	ACN MW, 130 °C, 60 min	No conversion
6	ACN MW, 150 °C, 60 min	No conversion
7	DMSO MW, 130 °C, 15 min	Trace
8	DMSO MW, 130 °C, 45 min	Degradation
9	NMP, Δ, 1.5 h	No conversion

[a] Ratio determined on the basis of ^{1}H-NMR.
[b] Isolated yield after pTLC.
[c] Ratio determined on the 280 nm UV-signals in the HPLC chromatogram.

As the thermal deprotection of the dipeptide **174** only provided the diketopiperazine **2** as a mixture of diastereomers, a switch was made to acid-mediated deprotection. Several protocols were evaluated on dipeptide **174**, which used TFA to accomplish Boc-removal (Table 14). The crude dipeptide was subsequently cyclized in the presence of a base namely, morpholine,[152] Et_3N, $NaHCO_3$[153] or 2-hydroxypyridine.[28]

Table 14: Boc removal in dipeptide 174 using TFA immediately followed by cyclization.



174 2a 187

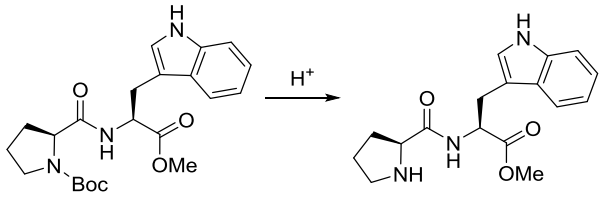
Entry	Reaction conditions	Result
1	1) 30% TFA CH_2Cl_2, 0 °C-r.t., 3 h 2) 35 equiv. morpholine CH_2Cl_2, r.t., 48 h	Mixture of **2a**/**187** 6/1[a] 1/1[b]
2	1) 30% TFA CH_2Cl_2, 0 °C-r.t., 30 min 2) 5% aq. $NaHCO_3$ MeOH, r.t., 2 h	Mixture of **169a**/**187**, 1/1[a] trace of **2a**
3	1) 30% TFA CH_2Cl_2, 0 °C-r.t., 3 h 2) 2 equiv. Et_3N CH_2Cl_2, r.t., 48 h	Mixture of **169a**/**187** 2/1[a]
4	1) 10% TFA CH_2Cl_2, r.t., 3 h 2) 0.2 equiv. 2-hydroxypyridine toluene, Δ, 12 h	Mixture of **169a**/**187**,[c] 3/1[b] trace of **2a**

[a] Ratio determined on the 280 nm UV-signals in the HPLC chromatogram.
[b] Ratio determined on the MS-signals in the HPLC-MS chromatogram.
[c] Compound **187** was not detected by UV absorption in the HPLC chromatogram.

In all cases, the Boc group was removed by treatment with TFA. The addition of $NaHCO_3$, Et_3N or 2-hydroxypyridine did not induce (complete) cyclization (entries 2-4). HPLC-MS analysis revealed the presence of the desired DKP **2a** after treatment with excess morpholine (entry 1). However, in all experiments, a side product was detected in HPLC-MS. The mass indicated the addition of a *tert*-butyl group to **2a**, which may be alkylated DKP **187**. This side product **187** would result from reaction of the indole moiety with the *tert*-butyl cation, which is released upon treatment of the Boc group with acid. This undesired side reaction was described by Lundt *et al.*[154] However, since the compound was never isolated, the exact structure of the side product (or position of the *tert*-butyl group in **187**) can not be elucidated.

To avoid this unwanted alkylation, 1,2-ethanedithiole was added as a scavenger to the reaction mixture (Table 15).[154] However, the undesired alkylated product **187** was still formed, even with excess scavenger.

Table 15: Boc removal using TFA in the presence of a scavenger.



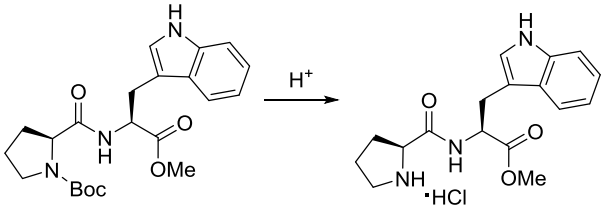
174 **169a**

Entry	Reaction conditions	Result
1	30% TFA, 1 equiv. 1,2-ethanedithiol CH_2Cl_2, 0 °C-r.t., 3 h	Mixture of **187**/**169a** 4/1
2	30% TFA, 2.5 equiv. 1,2-ethanedithiol CH_2Cl_2, 0 °C-r.t., 3 h	Mixture of **187**/**169a** 5/1

[b] Ratio determined on the MS-signals in the HPLC-MS chromatogram.

Alternatively, HCl was used to remove the Boc group. The HCl was formed *in situ via* reaction of acetyl chloride and methanol.[155] The deprotection was also performed in an ethyl acetate solution saturated with HCl. In both cases no alkylated side product **187** was detected on HPLC-MS analysis and the reaction produced **169a** in quantitative crude yield.

Table 16: Boc removal using HCl.



174 **169a**

Entry	Reaction conditions	Crude yield
1	5 equiv. MeOH, 5 equiv. acetyl chloride CH_2Cl_2, 0 °C, 3 h	Quantitative
2	HCl in EtOAc r.t., 5 h	Quantitative

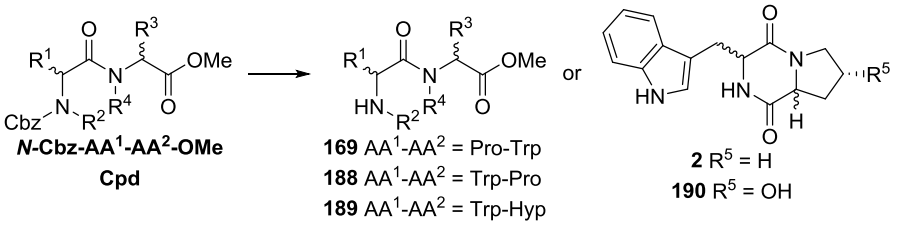
1.3.3. Cbz deprotection

The removal of the alternative N-Cbz protecting group was also investigated. The most straightforward way to accomplish this was through hydrogenolysis. Different hydrogen sources are possible (Table 17). For instance, ammonium formate was used. Complete conversion took place affording the DKP **2**, but the higher temperatures were detrimental to the preservation of the stereochemistry (entry 1). However, switching to a lower boiling solvent probably did not effectuate the release of hydrogen gas from ammonium formate so **180a** was retrieved (entry 2). Next, the reactions were performed under a hydrogen atmosphere. Again, the reaction did not result in full conversion in dichloromethane as a solvent, not even after prolonging the reaction time (entries 3 and 4). The reactions in methanol with $Pd(OH)_2/C$ or Pd/C both gave full conversion to the dipeptide

169a (entries 5 and 6). No spontaneous cyclization to the DKP **2a** was detected. The required reaction time was reduced by adding more catalyst (entry 7). The same conditions were applied to the other dipeptides (**181**-**182**), in which the order of amino acids was reversed (entries 8-13). Full conversions were also achieved. Under the present conditions the hydrogenolysis was a 'clean' reaction and no side reactions were detected.

The observation was made that the '*trans'*-isomeric dipeptides **181b**, **181c** and **182b**, undergo spontaneous cyclization to the DKP ring structure after Cbz-removal (entries 9, 10 and 13). This was not the case for the '*trans'*-dipeptide **169b** having a reversed order of amino acids (entry 7). Hence, the order in which the amino acids are coupled is of importance for allowing spontaneous cyclization.

Table 17: Cbz removal via hydrogenolysis.



Pro: R^1-R^2 or R^3-R^4 = -$(CH_2)_3$-
Trp: R^1 or R^3 = 3-indolylmethyl, R^2 or R^4 = H

Entry	Cpd	AA^1	AA^2	Reaction conditions	Result
1	**180a**	L-Pro	L-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C MeOH, Δ, 18 h	**2** *dr* = 1.5:1 *cis*:*trans*[a]
2	**180a**	L-Pro	L-Trp	4 equiv. $HCOONH_4$, 50 wt% Pd/C CH_2Cl_2, Δ, 18 h	**180a** Trace of **169a**
3	**180a**	L-Pro	L-Trp	5 bar H_2, 1 wt% Pd/C CH_2Cl_2, r.t., 18 h[156]	ratio **180a**:**169a** = 1:2[a]
4	**180a**	L-Pro	L-Trp	5 bar H_2, 1 wt% Pd/C CH_2Cl_2, r.t., 3 d	ratio **180a**:**169a** = 1:1[a]
5	**180a**	L-Pro	L-Trp	5 bar H_2, 1 wt% $Pd(OH)_2$/C MeOH, r.t., 18 h	**169a**
6	**180a**	L-Pro	L-Trp	5 bar H_2, 1 wt% Pd/C MeOH, r.t., 18 h	**169a**
7	**180b**	L-Pro	D-Trp	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 2 h	**169b**
8	**181a**	L-Trp	L-Pro	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 2 h	**188a**

9	**181b**	L-Trp	D-Pro	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 2 h	**2c**
10	**181c**	D-Trp	L-Pro	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 2 h	**2b**
11	**181d**	D-Trp	D-Pro	5 bar H_2, 2 wt% Pd/C MeOH, r.t., 3 h	**188b**
12	**182a**	L-Trp	L-Hyp	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 3 h	**189**
13	**182b**	D-Trp	L-Hyp	5 bar H_2, 5 wt% Pd/C MeOH, r.t., 3 h	**190b**

[a] Ratio determined on the basis of the MS signals in the HPLC-MS chromatogram.

The Cbz protecting group was preferred over the Boc protecting group as the hydrogenolysis was considered a milder procedure than the acidic deprotection. Moreover, the acidic deprotection provides the HCl salt of the deprotected dipeptide so the spontaneous ring closure of the L,D- and D,L-dipeptides would unlikely occur and extra base would be necessary to induce cyclization for all isomers.

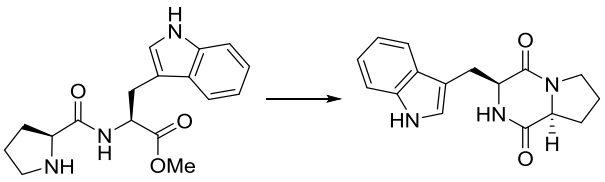
In view of the tentative idea of translating the DKP **2** synthesis into a continuous flow process, the Cbz protecting group may also be preferred over the Boc protecting group. The hydrogenolysis of the Cbz protecting group can more readily be transferred into a flow process. In contrast, the acidic deprotection of the Boc protecting group in a closed system of tubing does not readily allow the escape of the *tert*-butyl cation (as 2-methylpropene). This could again result in the formation of alkylated side products. In that case, further research into the addition of scavengers to prevent alkylation of the indole would be required. The synthesis of DKP **2** in a continuous process was not the topic of this dissertation.

1.3.4. Cyclization

The final step consisted of the cyclization of the dipeptides that did not spontaneously form the DKP. Table 18 shows the evaluated reaction conditions for dipeptide **169a**. The addition of hydroxypyridine did not effectuate the ring formation using DMF or methanol as solvents, and the starting material **169a** remained (entries 1-2).[57] The cyclized product **2a** was obtained after prolonged refluxing of **169a** in toluene, nevertheless some dipeptide **169a** was still present (entry 3).[28] Incomplete conversion was also found for the reaction in the presence of $NaHCO_3$ (entry 4).[153] The addition of piperidine resulted in the desired diketopiperazine **2a** at reflux (entry 5) and also at room temperature (entry 6), only a longer reaction time was needed in the latter case.[155]

Cyclization also occurred in the presence of morpholine (entry 7). However, repeated washing with 1 M HCl could not mediate complete removal of morpholine.[152] Although the desired product **2a** was obtained, the yields were still low. Finally, the dipeptide **169a** was dissolved in a solution of 7 M NH_3 in methanol.[84] The reaction was monitored with HPLC-MS until all starting material was converted. Recrystallization yielded the DKP in 63% yield as the sole product.

Table 18: Reaction conditions for inducing cyclization.

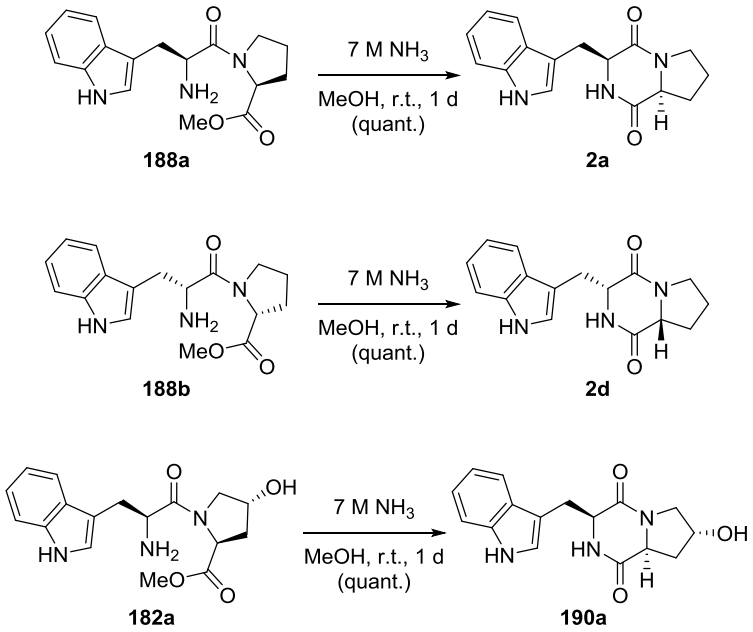


169a **2a**

	Reaction conditions	Yield 2a (%)
1	0.2 equiv. 2-hydroxypyridine DMF, 70 °C, 3 h	No conversion
2	0.2 equiv. 2-hydroxypyridine MeOH, Δ, 3 h	No conversion
3	0.2 equiv. 2-hydroxypyridine toluene, Δ, 12 h[28]	Not isolated
4	5% aq. $NaHCO_3$ MeOH, r.t., 2 h	Not isolated
5	3 equiv. piperidine DMF, Δ, 2 h	20[a]
6	3 equiv. piperidine DMF, r.t., 40 h	34[a]
7	Morpholine CH_2Cl_2, r.t., 48 h	37[a]
8	7 M NH_3 MeOH, r.t., 1 d	63[a]

[a] Isolated yield after recrystallization.

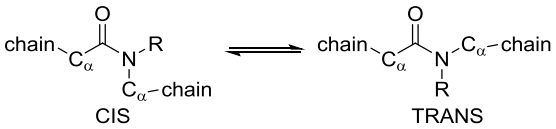
The same procedure was applied on dipeptides **188a**, **188b** and **182a**, which also provided the corresponding DKPs **2a**, **2d** and **190a** in quantitative yield (Scheme 32).



Scheme 32: Cyclization of 188 and 182a using ammonia in methanol. Crude yields for this step are depicted between parentheses.

A spontaneous ring closure took place for the D,L- and L,D-isomers of Trp-ProOMe, while an extra cyclization step was needed to convert the L,L- and D,D-isomers **188a** and **188b** to the DKP ring. No spontaneous ring formation occured with the L,D-isomer of Pro-TrpOMe **169** (*vide supra*, Table 8), so all isomers of **169** would require an extra cyclization step. Therefore the sequence starting from Cbz-Trp **155** was preferred.

The reason for the difference in cyclization tendency between the Trp-ProOMe and Pro-TrpOMe dipeptide esters can be attributed to the position of the proline amino acid in the dipeptide. An amide bond has a partial double bond character around the C-N bond as a result of delocalization of the electron lone pair on nitrogen. The partial double bond character results in hindered rotation of the amide bond and a distinction can be made between the *cis*- and *trans*-amide conformations (Scheme 33). In primary amino acids (R=H) an increased sterical hindrance is present in the *cis*-amide bond so the *trans*-amide isomer, which allows maximum separation, is favoured. However, proline forms an exception as a result of its secondary amine group. In proline ($R\text{-}C_\alpha\text{=}(CH_2)_3$) sterical hindrance will be present for both the *cis*- and *trans*-amide conformation resulting in a less pronounced energy difference between the two forms. Therefore, the amide bond in AA^1-Pro will more easily adopt a *cis*-conformation and a larger fraction will be present as the *cis*-isomer compared to primary amino acids.[157-158] This will increase the probability for cyclization of the dipeptide Trp-ProOMe as the *cis*-amide conformation is required for the formation of the diketopiperazine ring.



Scheme 33: *Cis*- and *trans*-amide conformations.

1.4. Collagen invasion assay

Several 2,5-diketopiperazines modulate tumor cell invasion *in vitro*. Some diketopiperazines **191**-**193** possessing anti-invasive properties are depicted in Figure 15.[159-161] Although the structures of these compounds differ considerably from each other, they all have the same piperazin-2,5-dione core. Since our group runs several projects on the development of anti-invasive molecules, we were intrigued to investigate our compounds in an *in vitro* model of invasion.

Moreover, the inhibition of invasion by HCCLM3 cells on treatment with the different stereoisomers of AIPZ **192** was configuration-dependent.[160] Hence, with all four isomers of **2** available, it was decided to investigate the influence of the different configurations of **2** on their inhibitory activity for invasion.

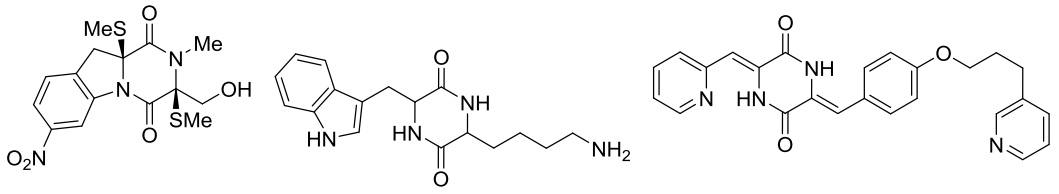


Figure 15: 2,5-Diketopiperazines with anti-invasive properties.

In a first set of experiments, the different isomers **2a-d** and **190b** were tested for their effectivity in inhibiting the invasive phenotype of cancer cells at the Laboratory of Experimental Cancer Research, Ghent University. This was done using a collagen assay on human N-Cadherin positive (PC3) and negative (DU145) prostate carcinoma cell lines.

A detailed protocol for the collagen invasion assay can be found in the reference of De Wever *et al*. (Figure 16).[162] Collagen type I gels as well as a single-cell suspension were prepared as described herein. Type I collagen is the main interstitial matrix component in solid tumors invasion. The tested compounds were diluted into DMSO and added to the single-cell suspension. This solution was deposited on top of the collagen type I gels. After incubation for 24 hours, invasive cells presenting invasive extensions into the collagen gel and non-invading cells were counted in 10–15 randomly

selected microscope fields. The results are reported as the mean invasion index, which is the ratio of the number of invasive cells over the total number of cells.

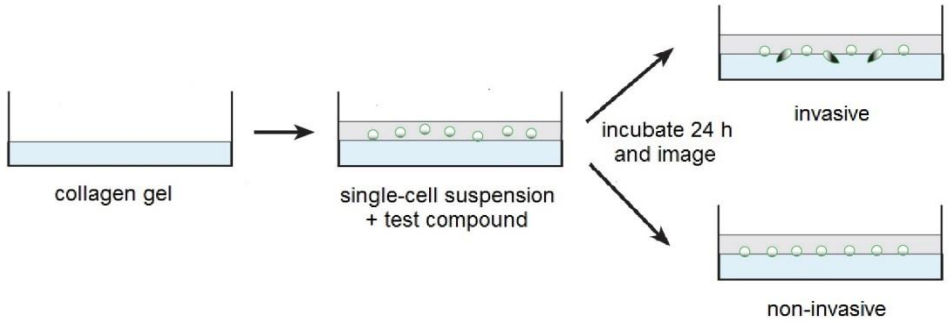


Figure 16: Schematic representation of the collagen invasion assay.

Isomers **2b** and **2d** were tested in four concentrations (0.01, 0.1, 1, 10 µM), while the other isomers were tested at 1 and 10 µM concentrations (Table 19). Cyclo(D-Trp, L-Pro) **2b** displayed a significant inhibitory effect for PC3 ($p<0.001$). For the DU145 cell line, the difference between the conditions was only borderline significant (Table 20). In both cases a dose-response behavior was noted (Chart 1). The outcome of this experiment was promising. In both cases a reduction of 75% of the mean invasion index was noted augmenting the concentration from 0.1 µM to 10 µM. However, as this experiment was only performed once, a confirmatory repeat experiment was necessary.

Table 19: PC3 cell line invasion index measured at 24 h for all compounds (in triplo), in the collagen invasion test.

	0.01 µM		0.1 µM		1 µM		10 µM	
Cpd	Inv. index	Mean SD	Inv. index	Mean SD	Inv. index	Mean SD	Inv. index	Mean SD
cyclo(L-Trp, L-Pro) 2a					25.31	8.58	23.26	9.76
cyclo(D-Trp, L-Pro) 2b	33.98	6.55	31.54	6.05	22.14	9.34	8.64	6.84
cyclo(L-Trp, D-Pro) 2c					21.47	10.55	24.23	9.49
cyclo(D-Trp, D-Pro) 2d	33.15	6.46	41.89	12.56	39.97	17.97	30.91	16.35
cyclo(D-Trp, L-Hyp) 190b					35.64	9.4	32.57	13.38

Table 20: DU145 cell line invasion index measured at 24 h for 2b and 2d (in triplo), in the collagen invasion test.

	0.01 µM		0.1 µM		1 µM		10 µM	
Cpd	Inv.index	Mean SD	Inv. index	Mean SD	Inv. index	Mean SD	Inv. index	Mean SD
cyclo(D-Trp, L-Pro) 2b	6.4	4.77	4.83	3.1	3.95	1.98	1.36	1.88
cyclo(D-Trp, D-Pro) 2d	25.86	6.19	21.64	4.74	17.94	7.69	15.51	3.94

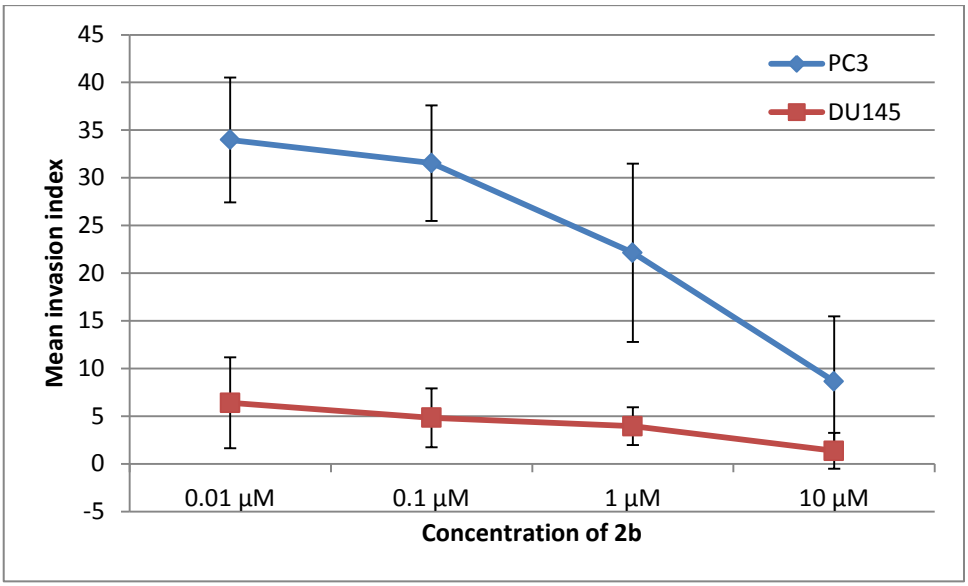


Chart 1: Mean invasion index for cyclo(D-Trp, L-Pro) 2b for the PC3 and DU145 cell line with 95% confidence interval, in the collagen invasion test.

In the second run of experiments, four different cell lines were chosen to explore cell line-dependent behavior: LNCaP as a non-invasive, N-Cadherine positive cell line, PTKI as a non-invasive, N-Cadherine negative cell line, PC3 as an invasive, N-Cadherine positive cell line and MDA-MB231 as an invasive, N-Cadherine negative cell line.

Overall, there was no significant inhibition by cyclo(D-Trp, L-Pro) **2b** for the PTKI cell line (Table 21, b), and the diketopiperazine **2b** also proved largely ineffective against invasion of MDA-MB cells (Table 21, d). For the LNCaP cell line, all values were significant, but there was no dose-response behavior (Table 21, a). Moreover, the difference with the DMSO control is too small to warrant further investigation.

Table 21: The invasion index for different cell lines measured at 24 h for cyclo(D-Trp, L-Pro) 2b (in triplo) in the collagen invasion test.

		DMSO control	0.1 µM		1 µM		10 µM	
	Cell line	Inv. index	Inv. index	p-value	Inv.index	p-value	Inv.index	p-value
a	**LNCaP**	0.407	0.245	< 0.001	0.272	< 0.001	0.275	< 0.001
b	**PTKI**	2.798	2.327	0.095	2.041	0.002	2.78	0.996
c	**PC3**	0.46	0.37	0.046	0.325	0.003	0.557	0.009
d	**MDA-MB231**	2.871	1.93	0.008	2.179	0.043	2.197	0.081

Unfortunately, contrary to the result obtained in the first experiment, the mean invasion index values against PC3 for compound **2b** at the tested concentrations fluctuate around the value for DMSO (Table 21, c and Chart 2). Hence, the anti-invasive effect of **2b** against invasion of PC3 cells in collagen could not be demonstrated in a reproducible manner within the timeframe of this work.

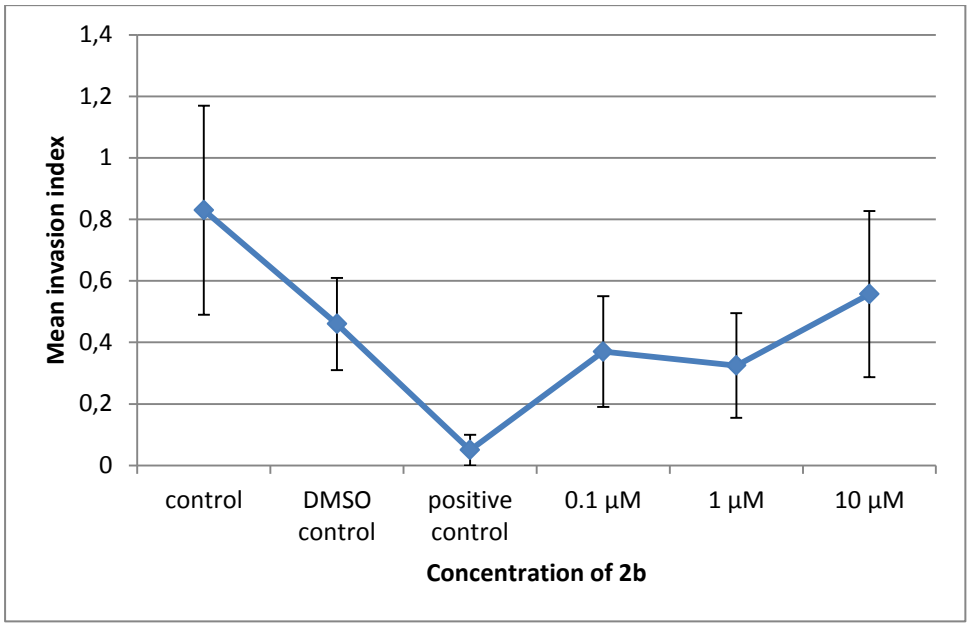


Chart 2: : Mean invasion index for cyclo(D-Trp, L-Pro) 2b for the PC3 cell line in the collagen invasion test.

In all, no striking or reproducible anti-invasive behavior could be noted for diketopiperazines cyclo(Trp, Pro) **2a-d** and cyclo(D-Trp, L-Hyp) **190b** in the collagen invasion assay against the evaluated cell lines.

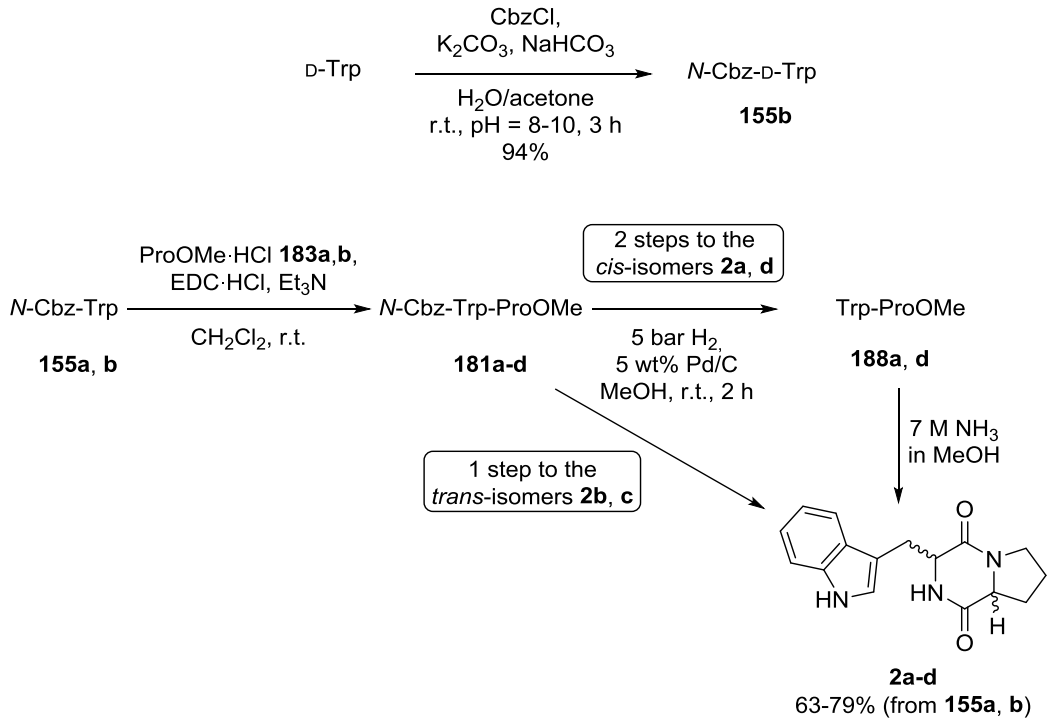
1.5. Conclusion

The phosphite-promoted condensation of unprotected amino acids did not provide the desired scaffold **2**, since only homocoupling of the Pro units occurred. The synthetic route based on the benzotriazole methodology required more steps than EDC-mediated coupling, resulting in lower overall yields. Overviews for the EDC- and benzotriazole-mediated coupling starting from D-Trp or L-Pro to accomplish the synthesis of cyclo(D-Trp, L-Pro) **2b** are shown in Scheme 35 and Scheme 36.

The procedure with EDC·HCl appears to be the best strategy of the ones investigated for the coupling of the amino acids, and eventually all four diastereomers **2a-d** could be synthesized in good yields (Scheme 34, Table 22). As a protecting group, the Cbz group was chosen. Carbobenzyloxy-protected D-tryptophan **155b** was obtained by treating D-tryptophan with benzyl chloroformate (CbzCl) in an aqueous solution of potassium carbonate and sodium bicarbonate.[140] The carbobenzyloxy (Cbz)-protected tryptophan **155** was coupled with proline methyl ester hydrochloride (ProOMe·HCl) **183** in the presence of EDC·HCl.[125] Subsequent hydrogenolysis of the crude dipeptides **181** with Pd/C under a H_2-atmosphere resulted in the deprotection of the dipeptides.

For the D,L- and L,D- dipeptides **181b** and **181c**, spontaneous cyclization towards the piperazin-2,5-dione **2b** and **2c** occurred. This was not observed for the analogous L,D-dipeptide **169b** having a reversed order of amino acids, which indicates the influence of the order in which the amino

acids are coupled and which led to the choice of starting the synthesis from Cbz-Trp. The cyclization of the other dipeptide isomers **188a** and **188b** into the respective diketopiperazines **2a** and **2d** required stirring with ammonia in methanol.



Scheme 34: Synthesis of the cyclo(Trp, Pro) 2 isomers.

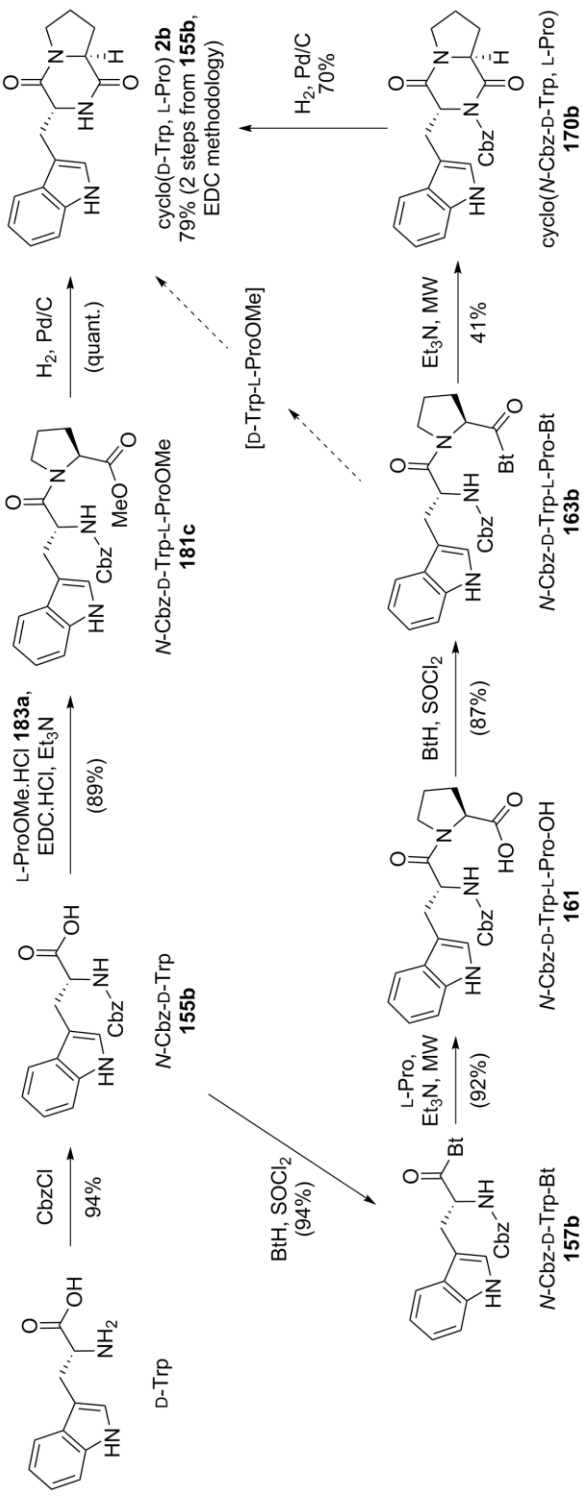
Table 22: Isomers of cyclo(Trp, Pro) 2 and cyclo(Trp, Hyp) 190.

Cpd		Yield (%)
2a	Cyclo(L-Trp, L-Pro)	70[a],[c]
2b	Cyclo(D-Trp, L-Pro)	79[b],[c]
2c	Cyclo(L-Trp, D-Pro)	74[b],[c]
2d	Cyclo(D-Trp, D-Pro)	63[a],[c]
190a	Cyclo(L-Trp, L-Hyp)	40[a],[d]
190b	Cyclo(D-Trp, L-Hyp)	40[b],[c]

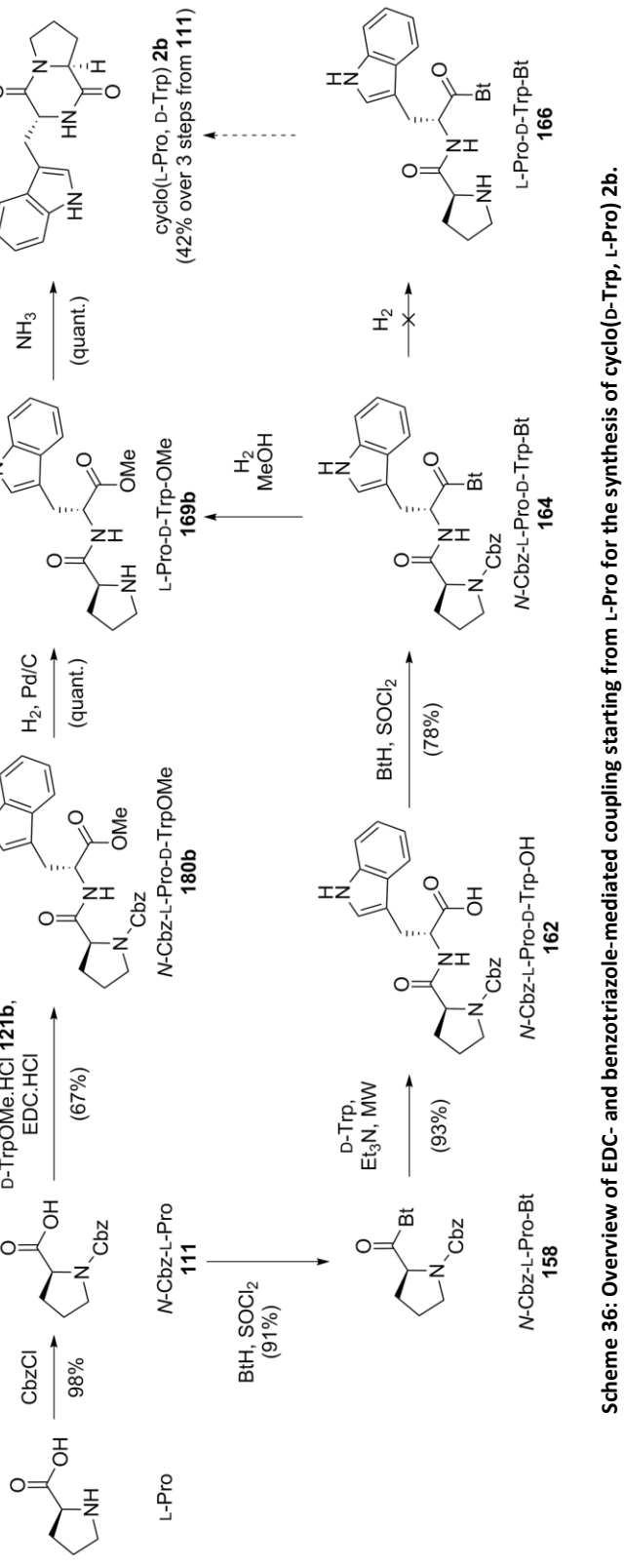
[a] Yield after 3 steps from **155**. [b] Yield after 2 steps from **155**.
[c] After recrystallization. [d] Purification with column chromatography.

The different isomers of cyclo(Trp, Pro) **2** and **190b** were evaluated for their inhibitory activity of invasion by cancer cells using the collagen invasion assay. In the first experiment a promising anti-invasive effect of **2b** against PC3 cells in collagen was detected. However, this effect could not be confirmed in a second series of experiments.

Having the diketopiperazine substrate in hand, it will be used as basic skeleton for the synthesis of analogues with a diverse substitution pattern to obtain a diverse set of functionalized brevianamide F analogues. The first goal was to synthesize annulated derivatives by means of a Pictet-Spengler reaction performed directly on DKP skeleton **2**.



Scheme 35: Overview of EDC- and benzotriazole-mediated coupling starting from D-Trp for the synthesis of cyclo(D-Trp, L-Pro) 2b. Crude yields are depicted between parentheses.



Scheme 36: Overview of EDC- and benzotriazole-mediated coupling starting from L-Pro for the synthesis of cyclo(D-Trp, L-Pro) 2b.

2. Modification of the diketopiperazine scaffold for the synthesis of brevianamide F analogues

2.1. Synthesis of annulated analogues *via* the Pictet-Spengler reaction

2.1.1. Introduction

A plethora of fungal secondary metabolites possess cyclo(Trp, Pro) **2a** as a basic skeleton and display attractive biological activities (*vide supra*). Therefore, these natural products are interesting lead compounds.

When comparing the different DKP-based inhibitors of cell proliferation, it can be concluded that the annulated derivatives fumitremorgin C **7** and demethoxyfumitremorgin C **91** are more active than their non-annulated counterparts, tryprostatin A **6** and B **85** (Table 2). Demethoxyfumitremorgin C **91** in particular has shown much promise in the development of anticancer drugs.[15, 90, 163] Similar to many of these fungal metabolites, demethoxyfumitremorgin C **91** also possesses a prenyl group that is connected to a 1,2,3,4-tetrahydro-β-carboline moiety. In synthetic chemistry, the Pictet-Spengler reaction, generally performed on arylethylamines,[164] can be used to introduce the 1,2,3,4-tetrahydro-β-carboline moiety.[165-166]

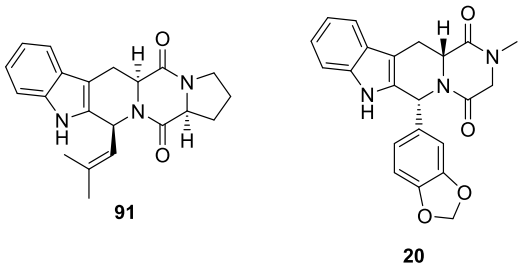
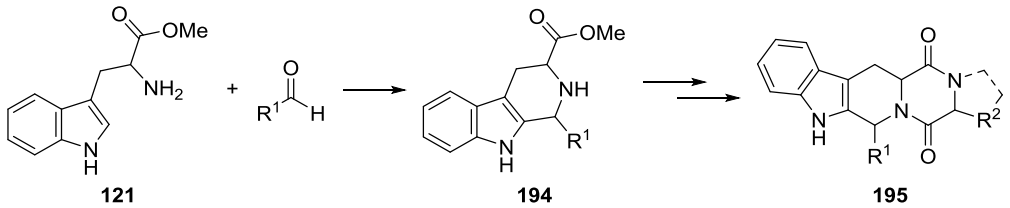


Figure 17: Demethoxyfumitremorgin C 91 and tadalafil 20.

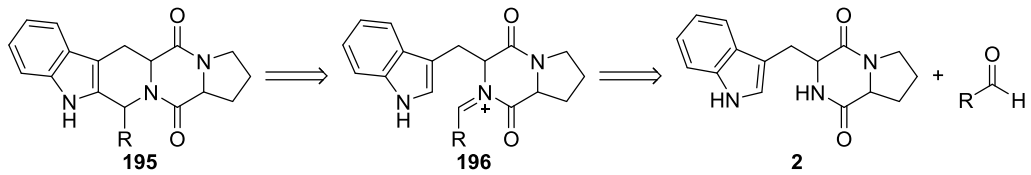
Demethoxyfumitremorgin C analogues have been synthesized and evaluated for their cytotoxic activity.[163] The different analogues were obtained by reacting tryptophan methyl ester **121** with different aldehydes and subsequently coupling each of the resulting tetrahydro-β-carbolines **194** with proline or other amino acids to obtain the annulated DKPs **195** (Scheme 37).



Scheme 37: Typical synthesis of DKPs 195 enclosing a tetrahydro-β-carboline moiety.

The Pictet-Spengler reaction is also a key step in the formation of tadalafil **20** (Figure 17). Similarly, when tadalafil **20** or analogues thereof are synthesized, the synthesis typically starts with a Pictet-Spengler condensation of a tryptophan ester **121** and an aldehyde to yield the tetrahydro-β-carboline **194**.[34, 167-170] The diketopiperazine ring **195** is only completed at a later stage. So, to evaluate the influence of different R groups in annulated derivatives of the general form **195**, different intermediates **194** have to be synthesized.

No articles have reported on the synthesis of analogues starting from the diketopiperazine scaffold **2**. Therefore, the goal of this chapter was to start to evaluate the Pictet-Spengler condensation on diketopiperazine **2** directly. This would reduce the number of required reactions when one wants to screen different R groups from different aldehydes while maintaining the same DKP skeleton (Scheme 38). The amide bond already present would lead to a N-acyliminium Pictet-Spengler reaction, which would proceed even faster than the normal Pictet-Spengler reaction (if the N-acyliminium intermediate **196** can be formed).[171]



Scheme 38: Retrosynthesis of annulated DKP 195 starting from DKP 2.

2.1.2. Pictet-Spengler reaction conditions lead to dimers

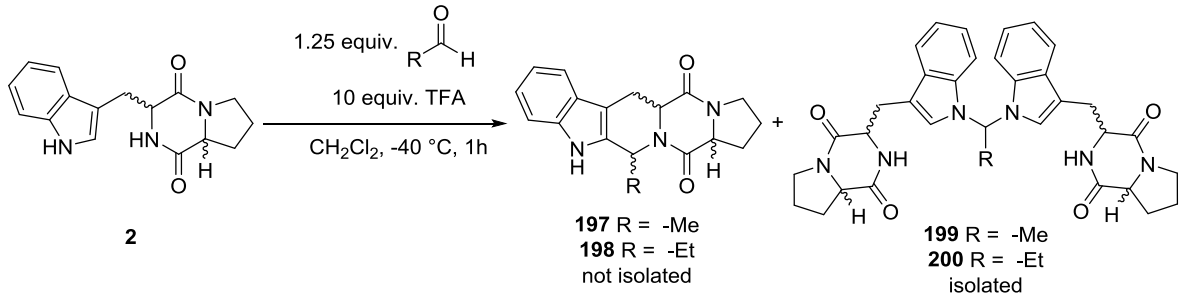
Initially diketopiperazine cyclo(Trp, Pro) **2** was subjected to Pictet-Spengler reaction conditions. To a solution of DKP **2** in dry dichloromethane was added the aldehyde in the presence of an excess amount of trifluoracetic acid at -40 °C (Scheme 39).[172] Both in the case of propionaldehyde and acetaldehyde, HPLC-MS analysis of the crude product revealed three signals. The mass of two peaks corresponded to the formation of the two isomers of the Pictet-Spengler product (**197** or **198**). Surprisingly, the HPLC-MS analysis showed a signal indicative of the formation of a high-in-mass, dimeric product next to the expected Pictet-Spengler product. However, during this research, a frequent observation was made that the DKPs studied, easily tend to form dimers in MS, so the presence of the high mass signal did not necessarily mean that such a dimeric product was present.

Monitoring the reaction with HPLC-MS revealed that after only five minutes both the peaks of the Pictet-Spengler isomeric products (**197** or **198**) as well as the signal for the high-in-mass product were detectable. As the reaction proceeded, the conversion of the three products augmented simultaneously. The relative proportion did not change after depletion of DKP **2**. From this

observation it was concluded that the product with high mass was not formed from the targeted Pictet-Spengler product.

Attempts to selectively crystallize one of the formed compounds failed. Crystallization with dichloromethane/diethyl ether only resulted in a residue that was enriched with the mass of the Pictet-Spengler product (**197** or **198**) and a mother liquor that was enriched with the high molecular weight compound. Repeating this procedure did not result in the isolation of one of the products.

Normal phase column chromatography with 2% methanol in dichloromethane as an eluent was used for the purification of the reaction mixtures after the reaction of cyclo(L-Trp, L-Pro) **2a** with acetaldehyde or propionaldehyde. Only one fraction could be separated that contained pure products. Mass spectral analysis evidenced the *m/z* values of 607 and 593, after the reaction with propionaldehyde and acetaldehyde, respectively. Thus, the presumed dimers were isolated. ^{1}H-NMR finally confirmed that dimeric structures **199** and **200** were indeed formed since the signals of the cyclo(Trp, Pro) moiety were present in duplicate.



Scheme 39: Pictet-Spengler reaction of cyclo(Trp, Pro) 2 with an aldehyde.

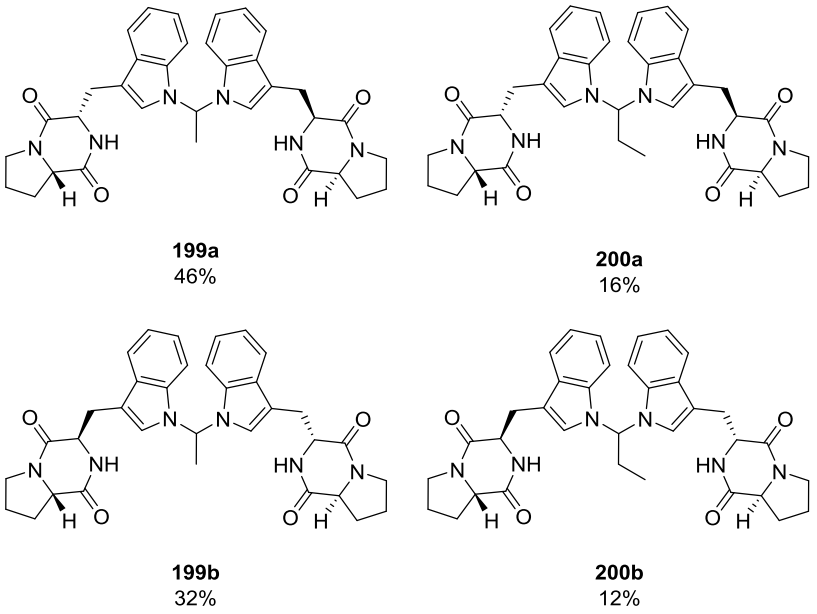
Beside the *cis*-isomer **2a**, also the *trans*-fused DKP cyclo(D-Trp, L-Pro) **2b** was investigated. The reaction of **2b** with propionaldehyde and acetaldehyde again resulted in the formation of three products (Scheme 39). The results were similar for both diastereomers **2a** and **2b** so the configuration of the amino acids has no effect on the reaction. Several attempts were made to isolate the different fractions through chromatographic methods. The compounds could not be isolated from pTLC. Normal phase column chromatography did not furnish the desired products, due to partial degradation on silica. However, through reversed-phase automatic chromatography, the dimers were eventually obtained.

The dimers are the result of the reaction of two equivalents of DKP with one equivalent of aldehyde. The isolated products were symmetrical. The presence of 2 NH signals around 8-9 ppm in ^{1}H-NMR misleadingly indicated the retention of the indole-NH. This led to the false assignment of two cyclo(Trp, Pro) units linked at the amide nitrogens. Unfortunately, this was only rectified at the end

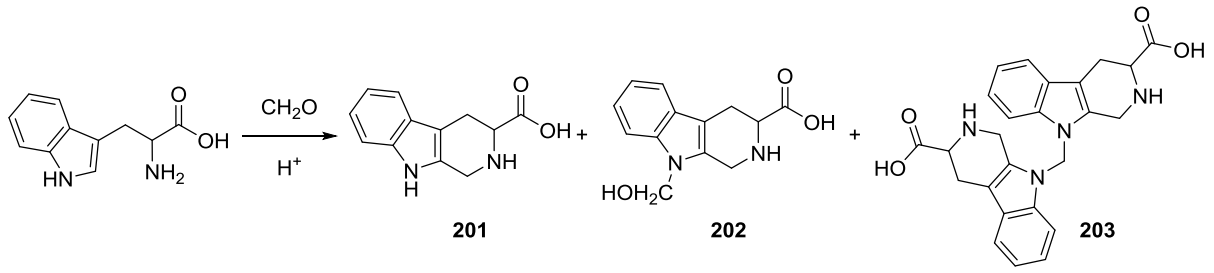
of this PhD by reanalyzing some products with the (new) 400 MHz NMR, which provided better spectra. Analysis of the COSY and HMBC spectra clearly demonstrated that these signals are in fact originating from the amide-NH, and have shifted significantly with respect to those in the monomeric substrates. The NH-singlets displayed coupling with the CH-9 (α to the carbonyl in tryptophan) in the COSY spectrum and they coupled with the carbonyl $C_{C=O}$-17 in the HMBC spectrum.

The structures of dimers **199** and **200** derived from cyclo(L-Trp, L-Pro) **2a** and from cyclo(D-Trp, L-Pro) **2b** with acetaldehyde and propionaldehyde are depicted in Figure 18. Since each time only one product was detected for the different dimers and taking into account the low reaction temperature (-40 °C) and short reaction time (1 h), it is assumed that the stereochemistry of the starting DKP did not change during the reaction. Difficulties with the purification resulted in low isolated yields of the dimers (Figure 18).

Figure 18: Dimeric products 199 and 200 obtained under Pictet-Spengler conditions.



An analogous reaction has been reported by Tourwé *et al.* when performing the Pictet-Spengler reaction with tryptophan and formaldehyde (Scheme 40).[173] After tryptophan had undergone the Pictet-Spengler reaction (**201**), two units were linked through a carbon-bridge connecting the indole-nitrogens (**203**) *via* intermediate **202**. The main difference with the previous results, is that the Pictet-Spengler reaction occurred first and the desired product was further converted into dimers.

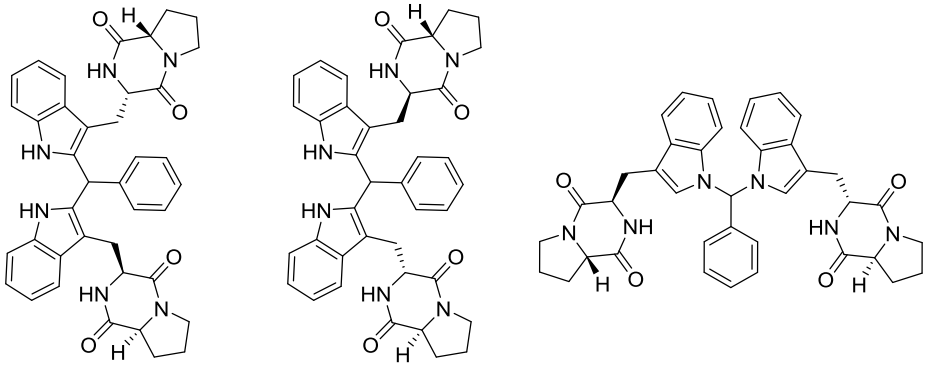


Scheme 40: Pictet-Spengler reaction of tryptophan with formaldehyde reported by Tourwé *et al.*[173]

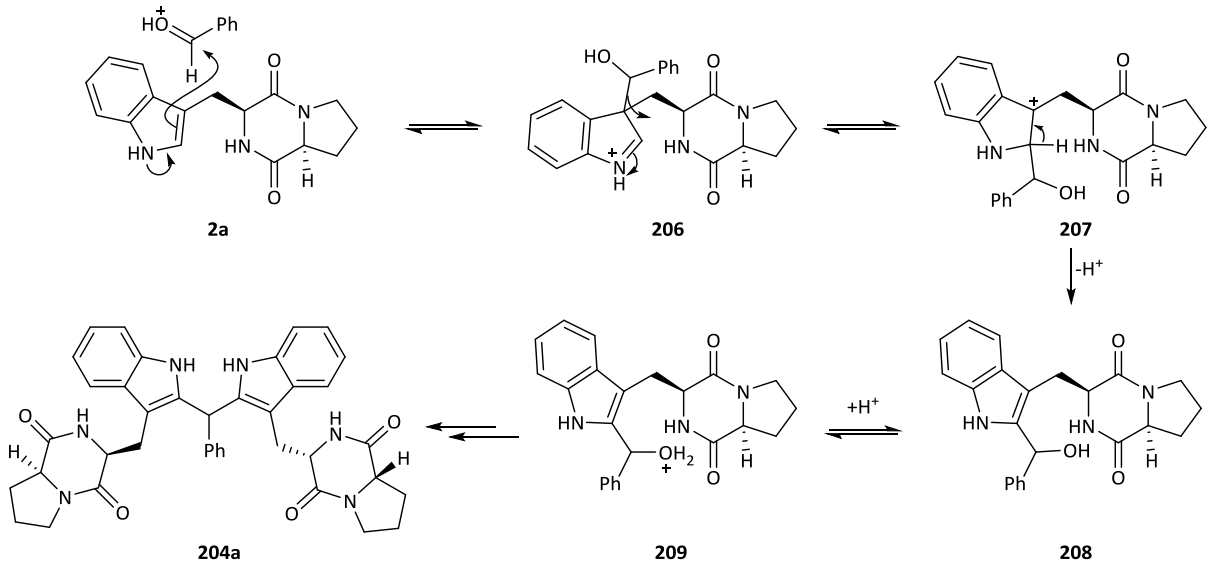
2.1.3. Pictet-Spengler reaction conditions with aromatic aldehydes

A difference in behavior of aliphatic and aromatic aldehydes was observed. The aliphatic acetaldehyde and propionaldehyde formed a bridge at the indole-nitrogens of the diketopiperazine ring. When the reaction was performed in the presence of benzaldehyde two products with a mass corresponding to the dimer were detected. Next to the expected dimer **205**, connected *via* the indole nitrogens, a dimer **204** with the connection via the C-2 of the indole moiety was also formed. In case of the L,L-isomer only **204a** could be isolated using normal-phase chromatography, but for the D,L-isomer both dimeric products **204b** and **205** were isolated using reversed-phase chromatography (Figure 19).

Figure 19: Dimeric products resulting from Pictet-Spengler conditions with benzaldehyde.



A possible explanation for the formation of these alternative dimers **204** can be found in the research of Jackson *et al.*[174] They investigated the electrophilic substitution at the 2-position of the 3-substituted indole nucleus and their findings are in agreement with the mechanism proposed in Scheme 41. The reaction starts with an initial attack at the 3-position of the indole (**206**) and is followed by a 1,2-shift of the substituent to the 2-position (**207**). The loss of a proton regenerates the aromatic indole moiety (**208**). The loss of water under acidic conditions (**209**) permits another molecule of diketopiperazine **2** to react in a similar fashion, providing the final dimer **204**.



Scheme 41: Possible mechanism for the formation of dimer 204a.

2.1.4. Screening different reaction conditions for aliphatic aldehydes

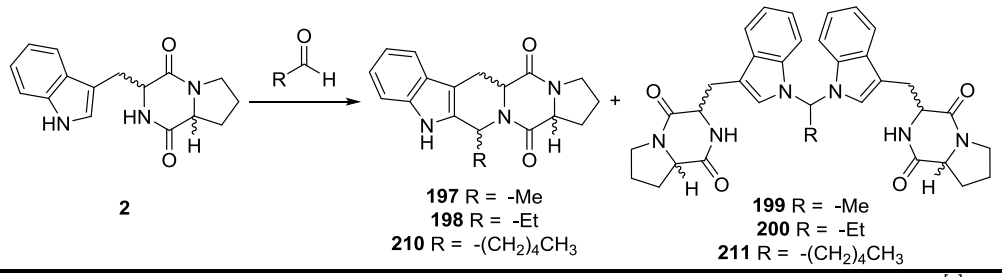
Several conditions were tested to enhance the formation of either the assumed Pictet-Spengler products (**197** or **198**) or of the dimers (**199** or **200**) (Table 23). The influence of reaction time, temperature and equivalents of reagents were screened.

The reaction conditions that were used initially, proved to be the best for the synthesis of the dimers (entries 1-3). A reaction time of one hour was preferred as a shorter reaction time gave incomplete conversion (entry 4). A longer reaction time presumably allowed the dimers to react further with aldehyde and products with a mass even higher than the mass of the dimers were detected (referred to as rest fraction in Table 23) (entry 5). At higher temperatures (0 °C to reflux) (entries 6-9), the fraction of these side products augmented as well. In entry 9, all starting material was converted to a mixture of many (ca. 10) non-polar, inseparable compounds, possibly degradation had occurred as a result of the high temperature. Lowering the temperature to -78 °C was promising as the largest fraction consisted of the assumed Pictet-Spengler product (entry 10).

Reduction of the amount of TFA was detrimental for the reaction and a higher reaction temperature was necessary to improve the conversion (entries 11-13). Reducing the equivalents of aldehyde (to 0.6 equivalents) mainly gave dimers but minor peaks of the other products remained visible (entries 14-15). Excess aldehyde (5 equivalents) surprisingly did not result in complete conversion. The crude mixture consisted for the largest part of dimers and no side products with high mass were detected (entries 16-17). Selective formation of one of both products could not be achieved by adjusting the amount of aldehyde used.

Reaction conditions involving other (Lewis) acids, instead of TFA, were screened as well (entries 18-24). Only the reactions with $BF_3 \cdot Et_2O$ gave conversion of the starting DKP, resulting in formation of the dimers.

Table 23: Screening of reaction conditions for the selective synthesis of Pictet-Spengler products or dimers.



Entry	Substrate	R	Reaction conditions	Result[a]
1	**2a**	Et	1.25 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1 h	**198a**/**200a** 1/1 (16%)
2	**2b**	Et	1.5 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1 h	**198b**/**200b** 2/3 (12%)
3	**2b**	Me	1.25 equiv. acetaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1 h	**197b**/**199b** 3/7 (32%)
4	**2b**	Et	1.25 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 50 min	**2**/**198b**/**200b** 4/50/46
5	**2b**	Et	1.25 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1.5 h	**198b**/**200b**/rest fraction 45/35/20
6	**2b**	Et	1.5 equiv. propionaldehyde, 5 equiv. TFA CH_2Cl_2, 0 °C, 10h -> r.t., 10 h	**2**/**198b**/**200b**/rest fraction 1/2/5/2
7	**2b**	Et	1.5 equiv. propionaldehyde, 5 equiv. TFA CH_2Cl_2, r.t., 20 h	**2**/**198b**/**200b**/rest fraction 1/2/3/4
8	**2b**	Et	1.5 equiv. propionaldehyde, 5 equiv. TFA CH_2Cl_2, Δ, 4 h	**2**/**198b**/**200b**/rest fraction 1/1/4/4
9	**2b**	Et	1.5 equiv. propionaldehyde, 5 equiv. TFA toluene, Δ, 1 d	Complex reaction mixture, complete conversion of DKP **2b**
10	**2b**	Et	1.25 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -78 °C, 2 h	**2**/**198b**/**200b** 1/5/4
11	**2b**	Et	1.05 equiv. propionaldehyde, 1.05 equiv. TFA CH_2Cl_2, -40 °C, 3 h -> r.t., 3 d	No conversion at -40 °C **198b**/**200b**/rest fraction 2/3/5
12	**2b**	Et	1.05 equiv. propionaldehyde, 1.05 equiv. TFA CH_2Cl_2, r.t., 9 d	**2**/**198b**/**200b** 2/3/5

13	**2b**	Et	1.05 equiv. propionaldehyde, catalytic TFA CH_2Cl_2, -78 °C, 2 h	No conversion
14	**2b**	Me	0.5 equiv. acetaldehyde, 10 equiv. TFA[2] CH_2Cl_2, -40 °C, 1 h	**197b**/**199b**/rest fraction 2/4/4
15	**2b**	Et	0.6 equiv. propionaldehyde, 0.6 equiv. TFA CH_2Cl_2, r.t., 22 h	**198b**/**200b**/rest fraction 1/4/5
16	**2a**	Et	5 equiv. propionaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1 h	**2**/**198a**/**200a** 2/3/5
17	**2a**	Me	5 equiv. acetaldehyde, 10 equiv. TFA CH_2Cl_2, -40 °C, 1 h	**197a**/**199a** 2/3/5
18	**2b**	$-(CH_2)_4CH_3$	4 equiv. hexanal, 2.2 equiv. $BF_3{\cdot}Et_2O$ CH_2Cl_2, -78 °C, 3 h[175]	**2b**/**210**/**211** 2/3/5
19	**2b**	Et	4 equiv. propionaldehyde, 2.2 equiv. $BF_3{\cdot}Et_2O$ CH_2Cl_2, -78 °C, 3 h[175]	**2b**/**198b**/**200b** 1/1/8 (10%)
20	**2b**	Et	1.5 equiv. propionaldehyde, 1.5 equiv. $Ti(OiPr)_4$ CH_2Cl_2, r.t., 4 d[176]	No conversion
21	**2b**	Et	1.2 equiv. propionaldehyde, 10 mol% $AlCl_3$, 1 equiv. BtH THF, r.t., 4 d[177]	No conversion
22	**2b**	Et	1 equiv. propionaldehyde, 0.1 equiv. H_3PO_4 toluene, Δ, 3 d[178]	No conversion
23	**2b**	Et	1 equiv. propionaldehyde, 0.1 equiv. pTsOH toluene, Δ, 3 d[179]	No conversion
24	**2b**	Et	1.5 equiv. propionaldehyde, 1/1 w/w H_3PO_4/P_2O_5 100 °C, 1 d[180]	No conversion

[a] Ratio obtained by integration of the 220 nm UV-signals in the HPLC chromatogram.

2.1.5. Rationalization and further attempts

The presence of dimeric structures means that an inter- instead of intramolecular reaction took place. The limited formation of Pictet-Spengler product will most probably result from an insufficient formation of the N-acyliminium intermediate from the DKP **2** contrary to the easier formation of an intermediate imine from the primary amine in tryptophan. Ducrot and Thal also aimed at performing a Pictet-Spengler reaction involving an N-acyliminium intermediate.[181] They found that this intermediate was not formed at a temperature below 0 °C. Since the reactions were performed at -40 °C in our case, the impeded formation of the N-acyliminium intermediate explains the smooth formation of the dimers, but it does not agree with the results at -78 °C where the assumed Pictet-Spengler product is the major fraction (entry 10).

Therefore, it is suggested that the nitrogen amide bond in the DKP is not nucleophilic enough to attack the aldehyde compared to the indole nitrogen. This also clarifies why at higher temperatures more side products are formed with masses exceeding the dimers. These products probably are dimers that have reacted with extra equivalents of aldehyde. Once the dimers are formed the alkyl bridge functions as a protecting group of the indole nitrogen leaving only the amide nitrogen to react with the aldehyde. This points out the need of a protecting group on the indole nitrogen for the reaction to succeed. Unfortunately, the assignment of the alkyl bridge connecting the two monomers through the indole nitrogens was not noticed sooner. Since it was assumed the amide was reactive enough, no protecting groups were tested in the timeframe of this PhD.

An appropriate protecting group would have to be acid resistant as TFA is used and would ideally enhance the nucleophilicity of the indole nucleus. Carbamate protecting groups (e.g. Boc or Cbz), sulfonyl derivatives (e.g. mesitylenesulfonamide) or *N*-trialkylsilylamines (e.g. TBDMS) do not comply. Preference would be given to N-alkyl or N-aryl derivatives (e.g. benzyl group). The most elegant solution would be to see if the alkyl bridge indeed functions as protecting group and let the dimers react further with the aldehyde to compounds analogous to **203** (Scheme 40). Subsequent hydrolysis of the aminal group as described for **203** by the group of Tourwé, should provide the desired Pictet-Spengler products.

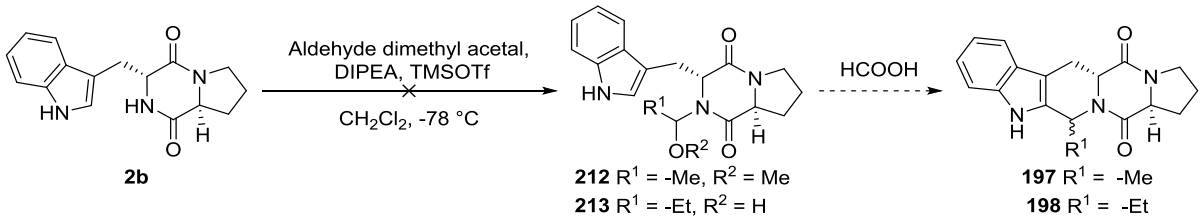
In addition, it can be speculated that the more rigid conformation of the DKP ring does not allow the expected intramolecular ring formation necessary for a Pictet-Spengler reaction.

On the other hand, a product was detected with a mass in agreement with the Pictet-Spengler product. This can indicate that the N-acyliminium is being formed to a limited extent. But, despite several trials to purify these products with normal-phase chromatography using different eluent

mixtures (ethyl acetate, dichloromethane/methanol, ethyl acetate/petroleum ether mixtures), the suspected Pictet-Spengler products were not isolated in pure form, so the structure could not be validated. Reversed-phase chromatography did result in the isolation of a fraction containing both of the isomers having the same mass of the Pictet-Spengler product. Their ^{1}H-NMR spectra showed too much signal overlap to assign a structure to the compounds.

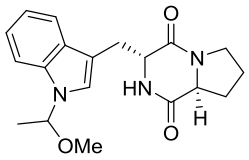
The low reactivity of the amide-nitrogen is probably the main reason why the side products are so easily formed. However, a similar reaction was demonstrated by Gonzalez *et al*.[182-184] They performed a Pictet-Spengler cyclization of a phenylalanine-based diketopiperazine with aldehyde dimethyl acetals in the presence of TMSOTf as the catalyst. The amide bond was first activated by adding TMSOTf to the piperazin-2,5-dione to form the O-trimethylsilyl derivative.

These reaction conditions were tested on cyclo(D-Trp, L-Pro) **2b** as a substrate. TMSOTf was added together with DIPEA in a first step to activate the amide bond (Scheme 42). The reaction was tested with propionaldehyde and with an acetal (1,1-dimethoxyethane) (Table 24). Following the procedure of Gonzalez *et al.*,[182] the mass of the intermediate hemiaminal ether **212** was detected in HPLC-MS. It was surprising that the hemiaminal **212** remained stable during the HPLC-MS analysis and did not readily undergo hydrolysis. However, subsequent treatment with formic acid returned the starting material **2b**. Analysis of the intermediate mixture after the first steps (entry 1) showed that the assumed intermediate **212** was in fact compound **214**. The indole nitrogen again proved to be more reactive. When propionaldehyde was used, no reaction took place.

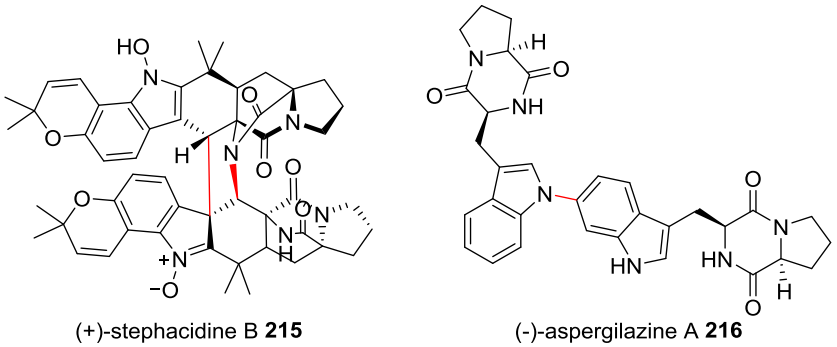


Scheme 42: Pictet-Spengler reaction with TMSOTf as catalyst.

Table 24: Pictet-Spengler reaction conditions using TMSOTf as catalyst.

Entry	R^1	R^2	Reaction conditions	result
1	-Me	-Me	a) 1 equiv. DKP **2b** 1.1 equiv. DIPEA, 1.2 equiv. TMSOTf CH_2Cl_2, -78 °C, 4 h b) 1.2 equiv. dimethoxyethane CH_2Cl_2, -78 °C, 4 h c) HCOOH, Δ	Mixture of **2b**/**214**/**199b**  **214** Recovery DKP **2b**
2	-Et	-H	a) 1 equiv. DKP **2b** 1.1 equiv. DIPEA, 1.2 equiv. TMSOTf CH_2Cl_2, -78 °C, 4 h b) 1.2 equiv. propionaldehyde, CH_2Cl_2, -78 °C, 4 h	No conversion, **2b** recuperated

Dimeric diketopiperazines based on a cyclo(Trp, Pro) skeleton also form a rare class of natural compounds. Amongst others this class includes stephacidine B **215**,[27, 30, 185] aspergilazine A **216**[186-187] (Figure 20) and brevianamide S **31**[68] and J **27**.[66] In general the connection between the two diketopiperazine units takes place through a linkage of the tryptophan units. Thus, here too the indole moiety is more reactive than the amide.



(+)-stephacidine B **215** (-)-aspergilazine A **216**

Figure 20: Dimeric diketopiperazines (+)-stephacidine B 215 and (-)-aspergilazine A 216.

Brevianamide S **31** exhibits selective antibacterial activity against Bacille Calmette-Guerin (BCG). Therefore, the antimicrobial activity of compounds **199b** and **200b** was tested by BCCM™/LMG (Laboratory of Microbiology, Ugent) against a panel of four bacterials strains: the Gram-negative *Escherichia coli* LMG 8063 and *Klebsiella pneumonia* LMG 2095, and the Gram-positive

Staphylococcus aureus LMG 8064 and *Bacillus substilis* LMG 13579. No antimicrobial effect was observed based on visual assessment of the turbidity caused by bacterial growth.

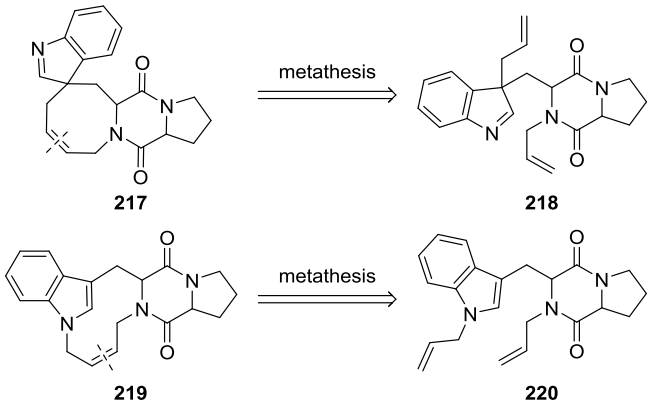
2.1.6. Conclusion

When subjecting a tryptophan-proline derived diketopiperazine to Pictet-Spengler conditions with aldehydes in the presence of trifluoracetic acid, dimeric products were isolated. These dimers are novel members of the class of dimeric diketopiperazines possessing a linkage between the two diketopiperazine units *via* an alkyl bridge between the indole nitrogens. In the case of aromatic aldehydes, another dimer was detected with the linkage at the C-2 carbon of the indole moieties. In all cases symmetrical dimers were obtained.

Products were also formed with a mass coinciding with the expected Pictet-Spengler product as evidenced by HPLC-MS analysis. Despite several trials, the desired compounds could not be isolated. Cyclo(Trp, Pro) as such is not suited as a starting point for the formation of annulated DKP derivatives *via* a Pictet-Spengler condensation reaction. The indole nitrogen was more reactive towards the aldehyde than the amide nitrogen, even after attempted activation of the latter by the addition of TMSOTf. A possible solution would be to introduce a protecting group on the indole nitrogen.

2.2. Ring formation through metathesis of allyl groups

The next objective consisted of the decoration of the diketopiperazine scaffold with allylic moieties analogous to the prenyl group, which is omnipresent in the natural products. Subsequently, metathesis reactions towards spiro or annulated derivatives would be pursued. By using unsaturated electrophiles such as allyl bromide, precursors (**218**, **220**) can be prepared which are suitable for transformation by metathesis reactions, with formation of compounds such as **217** and **219** (Scheme 43). Also dimerizations can be envisaged by metathesis reactions, which opens the way to prepare analogues of dimeric natural compounds.

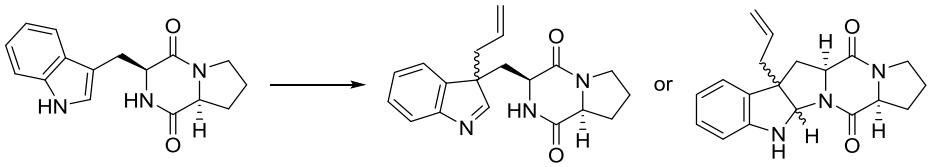


Scheme 43: Envisaged derivatives 217 and 219 through metathesis of allyl groups.

2.2.1. Introduction of the allyl group at C-3 of the indole

The synthesis of the spiro derivatives **217** required the introduction of an allylic moiety at the indole C-3-position. The formation of allylated compound **221** was aimed for. However, it was reasonable to expect that **221** would react intramolecularly and thus lead to the formation of an annulated derivative **222**. Different procedures based on palladium catalysis were tested on the scaffold cyclo(L-Trp, L-Pro) **2a** (Table 25).

Table 25: Procedures for the C-3 allylation of DKP 2a.



2a **221a** **222a**

Entry	Reaction conditions	Result
1	3 equiv. allyl methyl carbonate, 2.5 mol% $Pd_2(dba)_3$, 5 mol% P(2-furyl)$_3$ CH_2Cl_2, r.t., 1 d	No reaction
2	1 equiv. allyl alcohol, 5 mol% $Pd(PPh_3)_4$, 1 equiv. Et_3B THF, 50 °C, 5 h	**222a** (76%)
3	1 equiv. allyl alcohol, 5 mol% $Pd(PPh_3)_4$ polymer bound, 1 equiv. Et_3B THF, 50 °C, 5 h	No reaction

A procedure from Kagawa *et al*. was used for the palladium-catalyzed β-allylation of 2,3-disubstituted indoles, using allyl methyl carbonate as allylation precursor.[188] When these reaction conditions were applied on **2a**, no reaction took place (Table 25, entry 1).

Kimura *et al*.[189] used a Pd-Et_3B/allyl alcohol system for the C-3 selective allylation of indoles including L-tryptophan methyl ester **121a**. A similar, enantioselective procedure was elaborated by Trost *et al*.[190] The achiral conditions were applied on **2a** with full conversion of the starting material. The annulated product **222a** was formed as could be expected (Table 25, entry 2). Again difficulties arose for the isolation of the product. The separation of **222a** from the triphenylphosphine oxide byproduct could not be achieved by recrystallization, pTLC, column chromatography or filtration over silica plugs. Eventually, product **222a** was purified by pHPLC (Table 26). To prevent the release of the oxidized ligand, the reaction was repeated with polymer-bound catalyst (Sigma Aldrich, product number 511579, 0.5-0.9 mmol/g loading). However, no reaction was observed (Table 25, entry 3).

Remarkably, the reaction only proceeded with the catalyst from Sigma-Aldrich. No conversion occurred when the reaction was repeated with $Pd(PPh_3)_4$ from other suppliers, like Acros Organics or TCI. Serendipitously, the reaction was performed with the right catalyst the first time it was run.

The reaction conditions of Kimura *et al.* were applied on all the isomers of cyclo(Trp, Pro) **2**. The reaction was only successful for the *cis*-isomers **2a** and **2d** (Table 26). The *trans*-isomers **2b** and **2c** were not allylated.

Table 26: Yields for the isomers of allylated cyclo(Trp, Pro) 222.

Cpd	Starting compound	Yield (%)
222a	Cyclo(L-Trp, L-Pro)	76
222b	Cyclo(D-Trp, L-Pro)	No conversion
222c	Cyclo(L-Trp, D-Pro)	No conversion
222d	Cyclo(D-Trp, D-Pro)	68

Kimura *et al*. reported that only one diastereomer **223** was formed from L-tryptophan methyl ester **121a**. A similar stereochemistry is assumed at the newly formed stereogenic centers in **222a** and **222d** as only one product was detected with HPLC and ^{1}H-NMR for the conversion of **2a** and **2d** (Figure 21). This assumption was investigated with NOE experiments on compound **222a**.

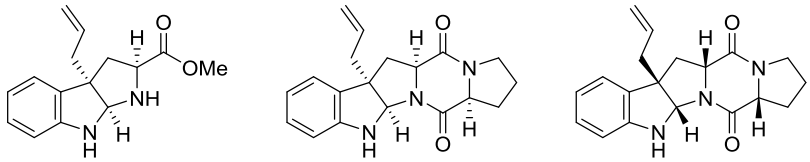


Figure 21: Novel C-3 allylated and annulated derivatives 222a and 222d.

NOE experiments clearly showed that H-2 and the allyl group are in each other's vicinity in space and thus have to be positioned at the same side of the molecule, as was expected (Figure 22). A small NOE was present between H-2 and the overlapping signals for H-9 and H-12, indicating that H-2 is situated at the same side of the molecule as H-9 and/or H-12. The stereocenters of tryptophan and proline are known to have the (*S*)-configuration and the allylation reaction does not influence the stereochemistry of the amino acids. Despite the fact that it is unclear whether H-2 is in the proximity of H-9, H-12, or both, it can be concluded that H-2, H-9 and H-12 are located at the same side of the diketopiperazine ring.

Additionally, a NOE was observed for both H-2 and H-19 with one and the same proton at position 8 (Figure 22). To determine how this proton was situated relative to H-9, the dihedral angle with H-9 was calculated from the $J_{H\text{-}H}$ coupling constant using the Karplus equation according to Haasnoot *et al*.[191-192] or Pachler.[193-194] From the ^{1}H-NMR spectrum, the coupling constants for the CH_2-8 protons were determined as 6.3 Hz and 11.1 Hz. The proton displaying a NOE with H-2 and H-19 has a coupling constant of 6.3 Hz. This corresponds to different angles, which were compared with a Dreiding model of the molecule. Therefore, it was concluded that the proton displaying a coupling constant of 6.3 Hz is H_a-8, positioned at a dihedral angle of 35-41° to H-9, and thus at the same side

as H-9. The proton H_b-8 has a coupling constant of 11.1 Hz and is positioned at an angle of 160-167°. These observations support the structure **222a**, where H-2, the allyl group, H_a-8 and H-9 are situated in each other's proximity on the same side of the molecule.

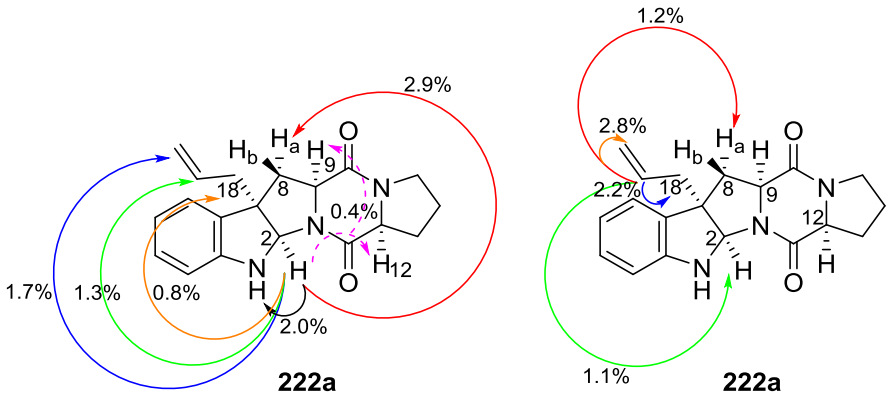


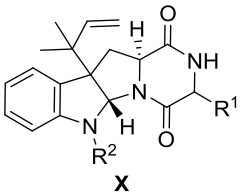
Figure 22: NOE by irradiating H-2 and H-19.

Table 27: Estimated torsion angles.

	Estimated torsion angles according to	
$^3J_{H-H}$ coupling constants	**Haasnoot *et al.*** [a]	**Pachler** [a]
6.3 Hz	**41°**, 135°, 230°, 324°	**35°**, 128°, 232°, 325°
11.1 Hz	**167°**, 198°	**160°**, 201°

[a] The values in bold are the possible angles according to the Dreiding model.

Compounds **222a** and **222d** are novel brevianamide derivatives that have a similar structure as several other tryptophan-based diketopiperazines such as fructigenine A **224**,[195] okaramine M **225**,[196] roquefortine D **226**[197] or brevicompanine B **227**, which are also annulated and possess an isoprenyl group at the C-3 (Figure 23).[198-199]



X

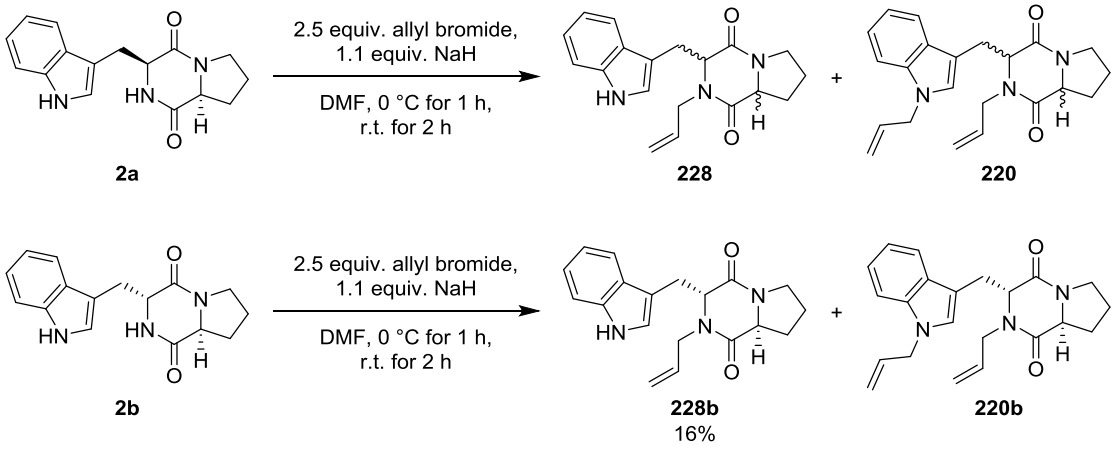
224 R^1 = (*S*)-Bn, R^2 = Ac, fructigenine A
225 R^1 = (*S*)-CH_2Ind, R^2 = Ac, okaramine M
226 R^1 = (*S*)-CH_2Im, R^2 = H, roquefortine D
227 R^1 = (*R*)-*i*Bu, R^2 = H, brevicompanine B

Figure 23: C-3 prenylated diketopiperazines.

2.2.2. Towards a spiro derivative

To prepare the desired spiro derivative **217** (Scheme 43), two allyl groups had to be introduced. In the first step it has been demonstrated that C-3 allylation is possible, but is accompanied by intramolecular ring formation through reaction of the amide with intermediate indolenine **221**. To prevent this spontaneous ring closure, the secondary amide bond was alkylated towards the tertiary amide. Since the diallylated product was the desired metathesis precursor, an allyl group was introduced.

With cyclo(Trp, Pro) **2** in hand, it was investigated whether selective monoallylation of the amide was possible in the presence of the free indole-NH. Reaction with one equivalent of base gave a mixture of mono- and di-allylated products, together with a significant amount of residual starting material (Scheme 44). When **2a** was reacted with base, epimerization occurred giving a complex mixture (**228**, **220**). This was not the case when **2b** was used, so **228b** could be isolated *via* pTLC. In **228b**, the allyl group was located at the amide position. The difference in pKa values of the amine and amide did not suffice to allow for a selective monoallylation, and the use of protecting groups was necessary.



Scheme 44: Attempted monoallylation of cyclo(Trp, Pro) 2a and 2b.

A Boc protective group was introduced on the indole amine of dipeptide **181a** (Scheme 45). Subsequent removal of the Cbz group from **229** and cyclization provided the corresponding diketopiperazine **230**. Deprotonation with a base in the presence of the electrophile allyl bromide resulted in the allyl derivative **231**, which had undergone partial epimerization.[121] Pure product **231** was isolated after column chromatography. Finally, selectively monoallylated DKP **228a** was obtained after removal of the Boc group from **231**.

The epimerization during alkylation of Boc-protected cyclo(L-Trp, L-Pro) **230** to provide **231**, can possibly lead to one of two thermodynamically more stable *trans*-isomers of **231**. To confirm the

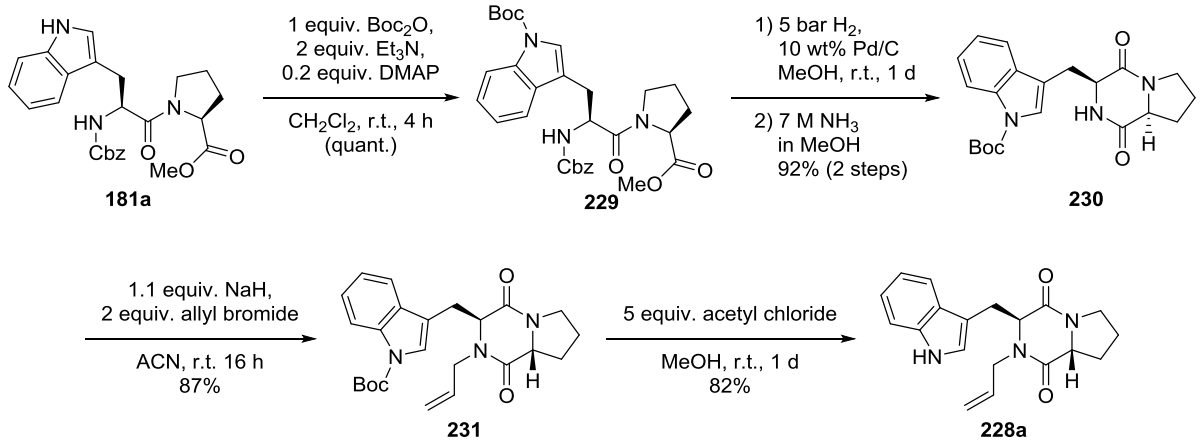
stereochemical assignment of the isolated allyl derivative **231**, the stereochemistry of **228a** was investigated.

The two possible *trans*-configurations of the diketopiperazine core that are under consideration are cyclo(D-Trp, L-Pro) or cyclo(L-Trp, D-Pro). However, the *trans*-isomer **228b** possessing the cyclo(D-Trp, L-Pro) ring was also obtained (*vide supra*) and the sign of the optical rotations of **228a** and **228b** are opposite. On the ground of the optical rotation, possible epimerization at the CH_α from tryptophan was excluded. Consequently, if **228a** was derived from epimerized **231** instead of its *cis*-isomer, the diketopiperazine ring would have epimerized at the CH_α from proline.

The possibility of **228a** being a *trans*-isomer was indicated by the low value of the CH_α from proline in ^{1}H-NMR (2.29 ppm). When looking at the different isomers of cyclo(Trp, Pro) **2a-d**, CH_α from proline (CH-12) has the same chemical shift as CH_α from tryptophan (CH-9) (around 4.1 ppm) for the *cis*-isomers **2a** and **2d**. In contrast, the *trans*-isomers **2b** and **2c** display a significant difference in shift for both CH_α's (2.8 ppm for CH-12 *versus* 4.1 ppm for CH-9) most probably as a result of shielding by the indole ring of tryptophan folding back over the diketopiperazine ring.[7, 200] However, the prenylated instead of allylated cyclo(L-Trp, L-Pro) analogue of **228a** has been reported by the group of R. M. Williams.[121] This compound also displays a shift of only 2.18 ppm for CH-12 and has been investigated by NOE experiments. The spectral data of **228a** and the prenylated compound from Williams are very similar apart from the methyl groups. The low chemical shift of CH-12 is thus possible and therefore will more likely result from the presence of the allyl group. Moreover, the procedure by the group of Williams[121] (as well as e.g. Scheme 9)[56-57] applies very similar reaction conditions and no epimerization at the proline CH_α was mentioned. This indicates that epimerization will not happen so readily at this position. In case of epimerization at CH-12, the absolute value of the optical rotation should be the same as for **228b** as they would then be enantiomers, which was not the case (75° versus 95°). Therefore, it was concluded at the time that **228a** and **231** were indeed the *cis*-isomers.

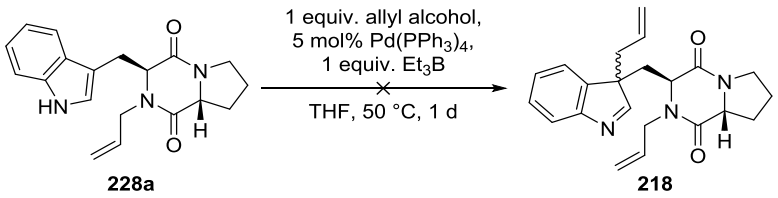
However, another report by Wang *et al.* also describes the formation of the *cis*- and *trans*-prenylated cyclo(Trp, Pro) scaffold.[163] This article provides other spectral data for the *cis*-product, which do not correspond to the data reported by the group of Williams. The ^{1}H NMR data for the *cis*-product provided by the group of Williams concurs better with the ^{1}H NMR data for the *trans*-product made by Wang *et al*. In retrospect, the data reported by Williams *et al*. are incorrect and will actually correspond to the *trans*-product. As a result, the wrong conclusion was initially made on the stereochemistry of **228a**, which will actually be the epimerized *trans*-product. This was corrected, but

no alternative synthesis was possible within the timeframe of this work. The difference in optical rotation values can be explained as the result of small impurities in the sample.



Scheme 45: Selective synthesis of monoallylated cyclo(L-Trp, L-Pro) 228a.

The method of Kimura *et al*. for the C-3 selective allylation of the indole, which worked on *cis*-DKP's **2a** and **2d**, was only applied on *trans*-DKP **228a**. As could be expected, no formation of **218** was observed (Scheme 46). No further investigations into the application of these reaction conditions on the other isomers or the use of other palladium catalysts were conducted within the timeframe of this work.

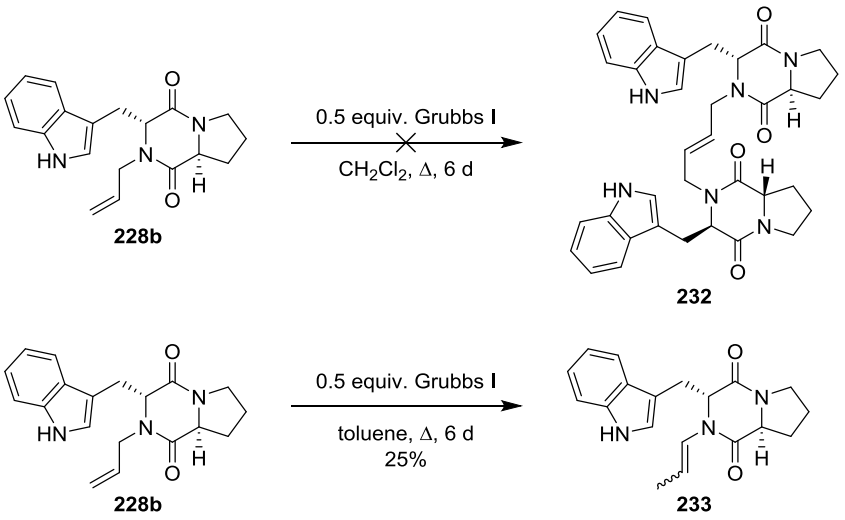


Scheme 46: Pd-catalyzed allylation of monoallylated 228a.

2.2.3. Towards a dimer and an annulated derivative

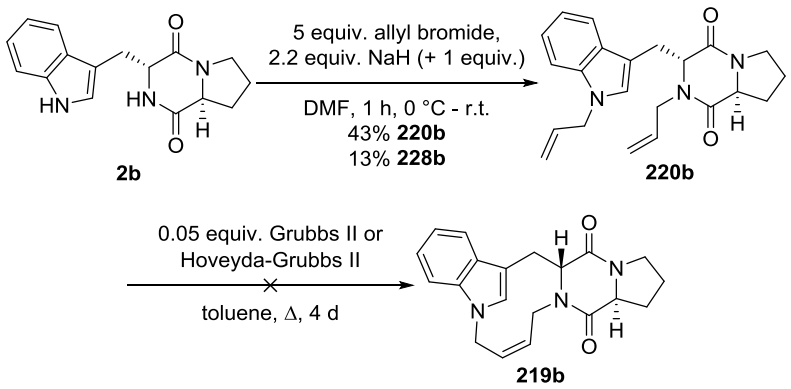
When the selective monoallylation of **2b** was attempted, mixtures of mono- and di-allylated products were obtained. Since the allylated products **228b** and **220b** were available, a brief investigation was made into their transformation to the corresponding dimers **232** or annulated derivative **219b** through metathesis. Unfortunately, all attempts towards the desired metathesis reactions failed.

Dimerization of mono-allylated **228b** using Grubbs I catalyst gave no conversion after refluxing in dichloromethane. In toluene, only isomerization of the double bond occurred (**233**, Scheme 47).



Scheme 47: Desired formation of dimers *via* metathesis from 228b.

Finally, the formation of the annulated compound **219b** starting from di-allylated compound **220b** was attempted. To get a better conversion to **220b**, more equivalents of base were added.[121] The conversion to **220b** improved, but still some monoallylated **228b** remained. Attempts towards ring formation of the di-allylated product **220b** gave no conversion, both with Grubbs II or Hoveyda-Grubbs II catalysts (Scheme 48). The lack of reaction can be the result of an unfavorably positioning of the two allyl groups to undergo metathesis. Presumably, conformational rigidity of the ring systems does not allow enough flexibility for the double bonds to align properly.



Scheme 48: Desired formation of annulated derivatives *via* metathesis from 220b.

2.3. Synthesis of annulated analogues *via* other electrophiles

2.3.1. Introduction

Many of the complex fungal metabolites containing the cyclo(Trp, Pro) skeleton are annulated (**7**), spiro-annulated (**86**) or have a bicyclic core (**3**) and thus possess an extra bridging structure connected to the diketopiperazine ring (Figure 24). Nature has most likely selected for the introduction of an extra ring system in these compounds to increase the conformational rigidity, which in its turn leads to a higher selectivity towards target proteins.

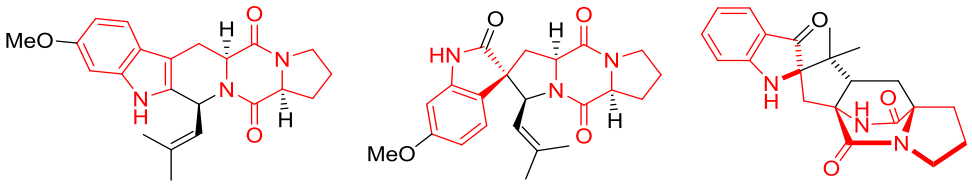


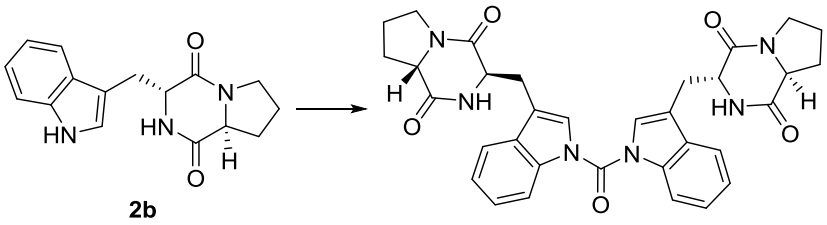
Figure 24: Fungal metabolites containing the cyclo(L-Trp, L-Pro) moiety.

To assess the possibility of introducing an extra ring via a carbonyl bridge between the amide-N and the indole group, difunctionalized electrophilic reagents including 1,1'-carbonylbis-1*H*-imidazole (CDI) and phosgene were reacted with cyclo(Trp, Pro). In the present chapter, an alternative bridging structure for the tryptophan-proline based diketopiperazine scaffold is described: the 3,5-bridged piperazine moiety.

2.3.2. Screening different electrophiles leads to the novel 3,5-bridged α-chloroamine

The synthesis of diketopiperazine **2** was performed using standard protocols (Scheme 34). In a first attempt to introduce an extra ring, cyclo(D-Trp, L-Pro) **2b** was reacted with CDI (Table 28). The reaction proceeded, very slowly, towards one product. Conversion remained incomplete even after several days (entries 1-2). Dimer **234** was obtained, which was a similar result to the earlier reported dimers derived from the Pictet-Spengler reaction (Figure 18). Due to the sensitivity of CDI to water, any residual (or introduced during sampling) moisture in the solvents will result in loss of some CDI, which may have caused the limited conversion. Therefore, excess CDI was added to improve conversion and to possibly speed up the reaction (entry 3), but no improvement was noted.

Table 28: Screening of reaction conditions for the synthesis of compound 234.



2b **234**

Entry	**Conditions**	**Conversion to 234**[a],[b]
1	1.1 equiv. CDI, 2 equiv. Et_3N CH_2Cl_2, Δ, 3 d	60% (15%)
2	0.6 equiv. CDI, 1 equiv. Et_3N CH_2Cl_2, Δ, 7 d	40% (8%)
3	10 equiv. CDI, 3 equiv. Et_3N CH_2Cl_2, Δ, 9 d	50%
4	10 equiv. CDI, CH_2Cl_2, Δ, 5 d	No conversion
5	1.1 equiv. CDI, 3 equiv. Et_3N CH_2Cl_2, Δ, 5 d	80% (65%)
6	1.1 equiv. CDI, 2 equiv. Et_3N CH_2Cl_2, MW: 60 °C, 1 h	35% conversion to unidentified compound[a]
7	1.1 equiv. CDI, 2 equiv. Et_3N CH_2Cl_2, MW: 100 °C, 1 h	60% conversion to unidentified compound[a]
8	1.1 equiv. CDI, 2 equiv. Et_3N CH_2Cl_2, MW: 100 °C, 2 h	35% conversion to unidentified compound[a]
9	1.8 equiv. Me_2CO_3, 3.6 equiv. DIPEA CH_2Cl_2, r.t., 1 d	No conversion
10	1.8 equiv. 1,3-dioxolan-2-one 3.6 equiv. DIPEA CH_2Cl_2, r.t., 1 d	No conversion
11	1.2 equiv. $ClCO_2Me$, 1.2 equiv. DIPEA CH_2Cl_2, r.t., 5 d	No conversion

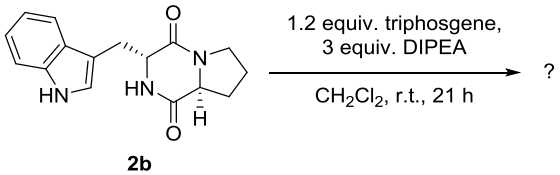
12	1. 5 equiv. $(CO)_2Cl_2$, 3 equiv. Et_3N CH_2Cl_2, r.t., 4 h	Complex reaction mixture

[a] Conversion obtained by integration of the 220 nm UV-signals in the HPLC chromatogram. [b] Isolated yields are indicated in parentheses.

In the absence of Et_3N, no reaction took place and DKP **2b** could be retrieved (entry 4). As the base proved to play a vital role in the reaction, excess base was also evaluated and a good conversion was achieved (entry 5). In an effort to speed up the reaction, it was performed under microwave conditions at elevated temperature and pressure. Generally, reactions proceed faster using microwave synthesis simply because they are conducted at higher temperatures.[201] However, no conversion to the desired dimer was achieved (entries 6-8). Nonetheless, partial conversion of the starting material to another compound was detected by HPLC-MS. Unfortunately, despite several trials, this compound could not be isolated for identification using pTLC with several eluent mixtures, due to degradation on the stationary phase.

Some other electrophiles were screened. Carbonates gave no reaction with DKP **2b** under the current conditions (entries 9-11). The reaction with oxalyl chloride was irreproducible and mixtures of several polar and non-polar compounds were obtained (entry 12).

Cyclo(D-Trp, L-Pro) **2b** was reacted with triphosgene in the presence of *N,N*-di-isopropylethylamine (DIPEA), to assess the possibility of introducing a carbonyl bridge between the amide-N and the indole group (Scheme 49).[202] However, another, unknown compound was formed during this reaction and isolated *via* pTLC. Mass spectrometry analysis of the unknown compound showed two molecular ion peaks (*m/z* 302 and 304, $[M + H]^+$) with peak heights in a 3:1 ratio, which indicated the presence of a chlorine atom in the structure. The molecular mass of the DKP starting materials **2** is 283 Da. Replacing a hydrogen for a chlorine atom exceeds the recorded mass (M-H+Cl : 283-1+35 = 317). Hence, it was concluded that an oxygen atom was expelled from DKP **2** (M–O+Cl : 283–16+35 = 302).

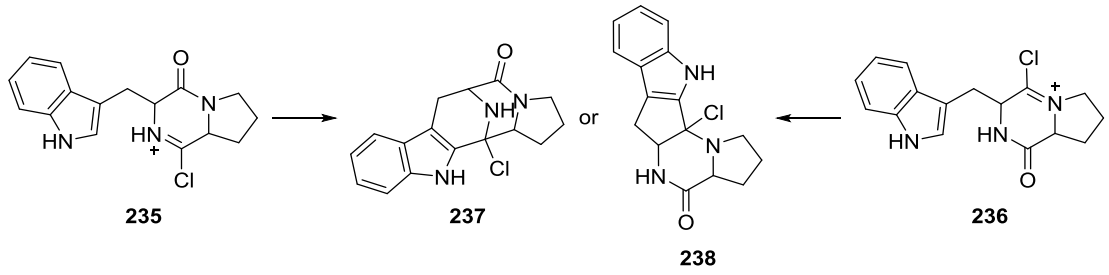


2b

Scheme 49: Reaction of cyclo(D-Trp, L-Pro) 2b with triphosgene.

Phosgene is known to react with amides to afford an imidoyl chloride. However, this intermediate (**235** or **236**, Scheme 50) would not be stable and would have reacted with the indole moiety. ^{1}H-

NMR studies indeed indicated that reaction had taken place at C-2 of the indole, since the signal of that proton had disappeared. The proposed structures (**237** and **238**) for the unknown compound included the remarkable feature of an α-chloroamine. Since the reaction could have taken place at either amide function, two possible structures remained: **237** and **238** (Scheme 50).



Scheme 50: Structural elucidation of the product from reaction of 2 with triphosgene.

X-ray analysis was envisaged to solve the structure and the compound was dissolved in a minimal volume of CH_2Cl_2. Slow evaporation of the solvent provided several prism-shaped crystals. Using X-ray analysis, the structure was confirmed to be pentacyclic α-chloroamine **237b** (Figure 25). Indeed, a C-C bond had been formed between the C-2 of the indole moiety and the carbonyl carbon originating from proline. From this analysis, it was clear that the relative stereochemistry of the DKP was retained during the reaction. Surprisingly, two different crystal packings, both determined as 'block' shaped, were detected for two separate repetitions of crystallizations performed in the same manner. However, the speed of evaporation may have differed depending on the temperature in the laboratory.

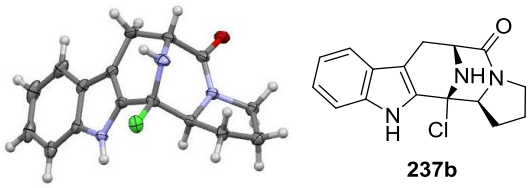


Figure 25: X-ray analysis of the newly formed pentacyclic α-chloroamine 237b (thermal ellipsoid contour probability level 50%).

In the first type, hydrogen bridges were present in the crystal packing between the indole-NH of one molecule and the C=O carbonyl of another molecule (Figure 26).

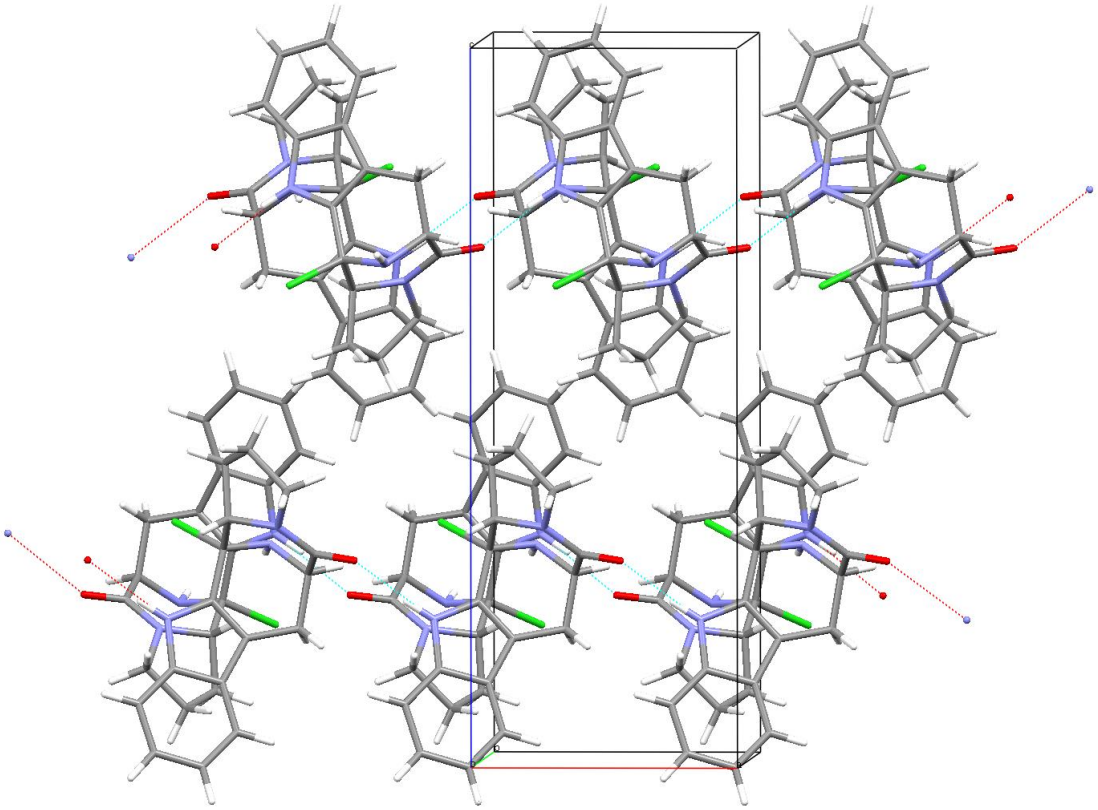


Figure 26: First type of crystal packing of 237b.

The second type of crystal was composed of the same compound **237b** , but the unit cell and packing were different. In this case, there were 3 molecules building up the asymmetric unit (Figure 27). Here too, hydrogen bridges in the crystal packing were detected between the indole NH of one molecule and the C=O carbonyl of another molecule. When the three molecules building up the asymmetric unit are fitted, it can be seen that one of the molecules is inverted (Figure 28). The indole NH is oriented to the opposite side of that in the other two molecules.

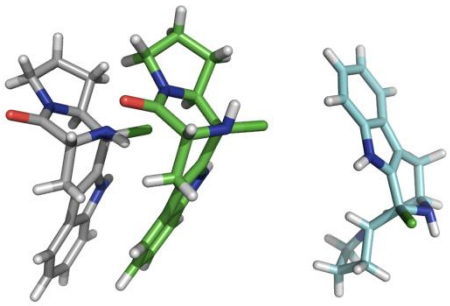


Figure 27: Unit cell of second type of crystal packing of 237b.

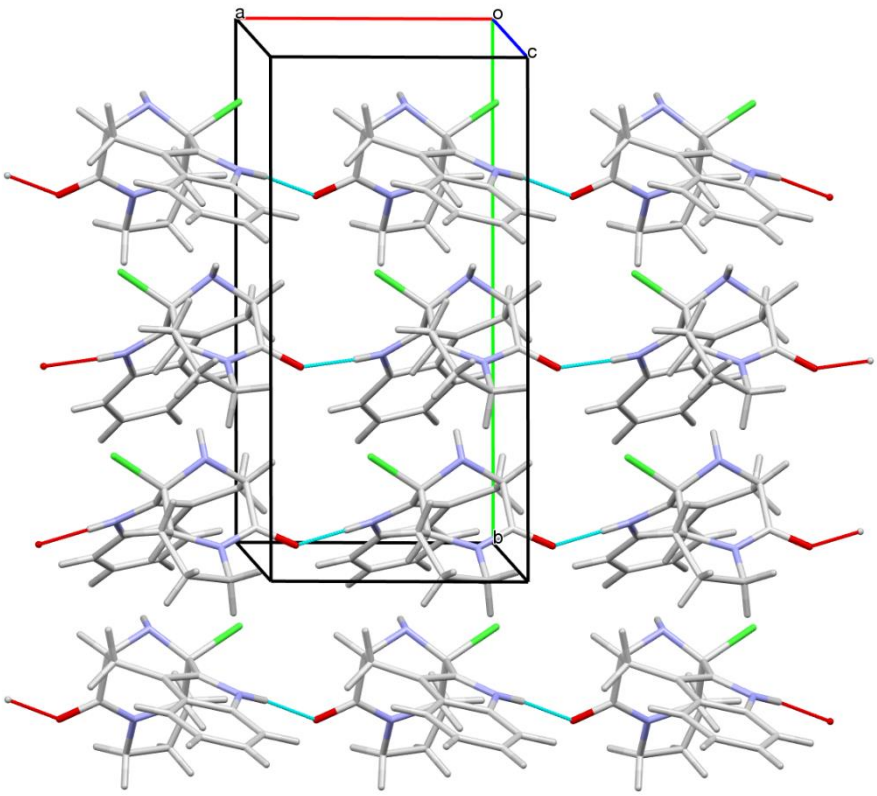
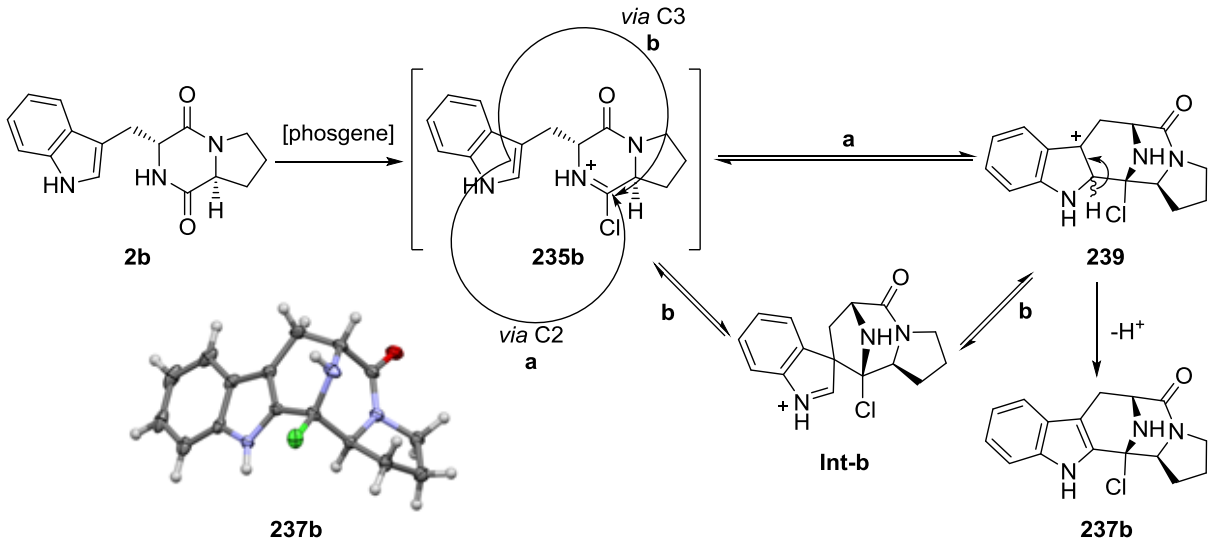


Figure 28: Second type of crystal packing of 237b.

2.3.3. Mechanistic discussion for the formation of the α-chloroamine

It is assumed that formation of the α-chloroamine **237b** resembles a Vilsmeier-Haack reaction. We propose that the reaction of tri- (or di)phosgene with the amide function of **2b** leads to an intermediate imidoyl chloride **235b** (Scheme 51), which is followed by an electrophilic aromatic substitution of the indole moiety with the imidoyl chloride. The latter transformation could be started *via* nucleophilic attack by either the C-2 (pathway a), or the C-3 atom of the indole function (pathway b). C-3 attack would lead to an intermediate spiroindolenine (**int-b**), which undergoes a 1,2-shift giving rise to product **239**, which can then readily lose a proton affording α-chloroamine **237b**.



Scheme 51: Reaction of piperazin-2,5-dione 2b with tri- (or di)phosgene to α-chloroamine 237b.

Although most literature precedents suggest that the more favorable 6-endo-trig cyclization (pathway a)[203-204] may be preferred over the less favorable 5-endo-trig cyclization (pathway b), evidence for the formation of a spiroindolenine (**int-b**) intermediate can be found as well.[205-207] To investigate the mechanism of the observed reaction, both pathways were studied by density functional theory (DFT) calculations performed at the Center for Molecular Modeling, Ghent University, Belgium. Gibbs free energy profiles for the involved transformations are shown in Figure 29. For both pathways, two different transition states - *exo* and *endo* - were found, leading to two different protonated α-chloroamines **239**, which both give rise to the α-chloroamine **237b** after deprotonation.

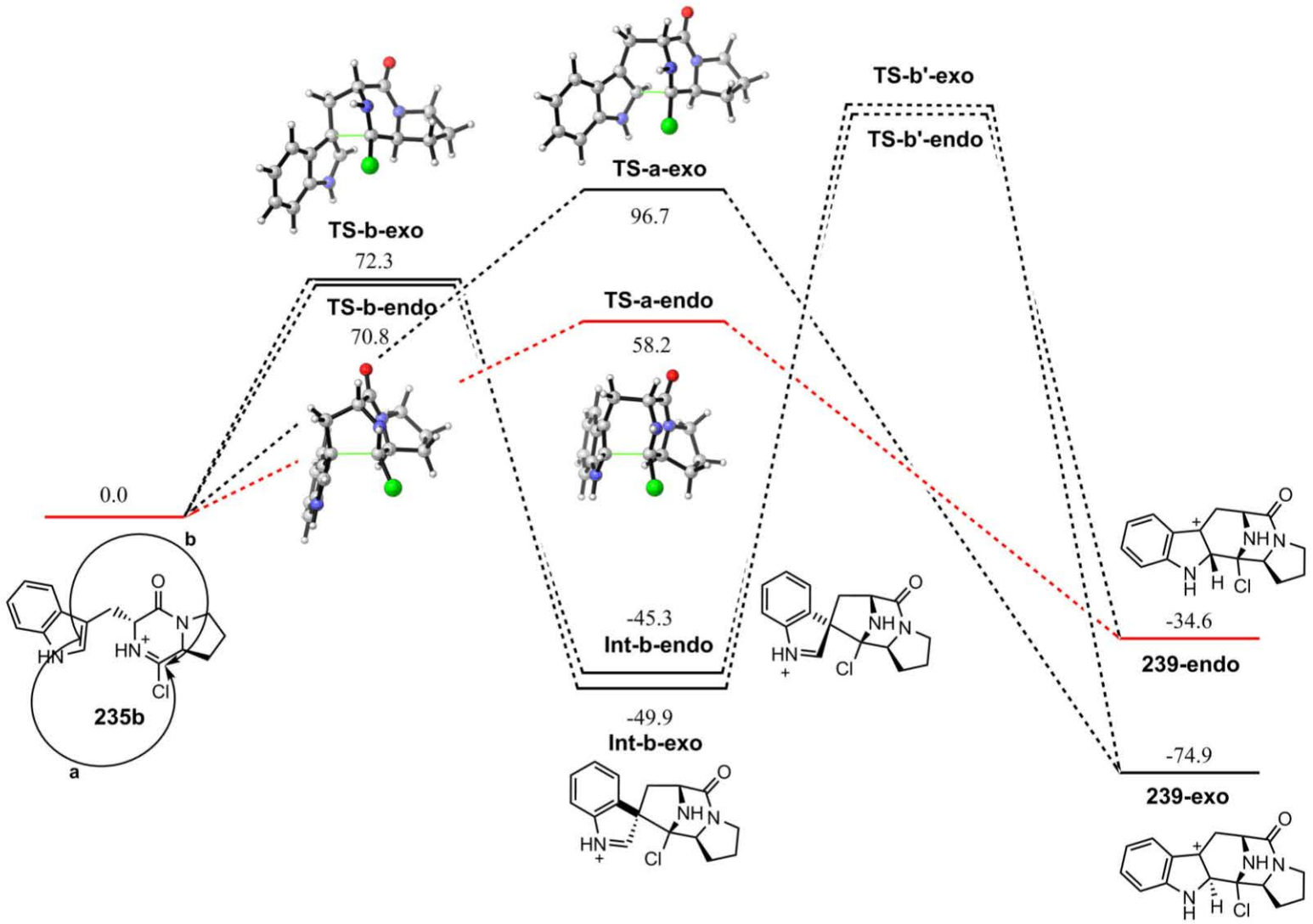


Figure 29: Free energy profiles (kJ/mol) for the reaction of the intermediate imidoyl chloride 235b to the protonated α-chloroamine 239 (PCM (ε = 8.93) M06-2X/6-31+G(d,p)).

Free activation energies show that pathway a (direct attack by the C-2 atom) is the kinetically preferred route - *via* **TS-a-endo** - ($\Delta G^{\ddagger}$ = 58.2 kJ/mol at the M06-2X/ 6-31+G(d,p) level of theory). Product **239-endo** is the kinetically preferred compound and is quickly deprotonated towards the neutral α-chloroamine **237b** upon formation. Therefore, equilibration of **239-endo** *via* **235b** to the thermodynamically preferred spiroindolenines **Int-b** (pathway b, *via* C-3 attack) is not feasible. Moreover, various attempts to model the necessary 1,2-shift (*via* **TS-b'**) between the intermediate spiroindolenine **int-b** and the protonated product **239** failed, as was previously found by Maresh *et al*. as well.[204] Presumably, if the intermediate spiroindolenine **int-b** would be formed, it would not undergo a 1,2-shift with formation of the protonated α-chloroamine **239** under the current reaction conditions, since this would imply the involvement of a high-energy intermediate. It can thus be concluded that α-chloroamine **237b** is most likely formed *via* direct attack by the C-2 atom of the indole function and not *via* C-3 attack followed by a 1,2-shift.

2.3.4. Improving conversion towards the α-chloroamine

The reaction suffered from low yields, partly due to incomplete conversion of cyclo(D-Trp, L-Pro) **2b** (Table 29). To improve the conversion, longer reaction times (entries 1-3) and higher temperatures (entry 4) were evaluated. A run with a larger excess of triphosgene was also attempted (entry 5). These modifications did, however, not lead to a dramatic increase in formation of **237b**. The addition of more base was detrimental to the reaction (entry 6).

Table 29: Reaction conditions tested on cyclo(D-Trp, L-Pro) 2b for the synthesis of 237b.

Entry	**Electrophile**	**Base**	**Solvent**	**T**	**t**	**Ratio 2b/237b**[a]
1	1.2 equiv. triphosgene	3 equiv. DIPEA	CH_2Cl_2	r.t.	21 h	1/2 (16%)
2	1.2 equiv. triphosgene	3 equiv. DIPEA	CH_2Cl_2	r.t.	2 d	1/3 (24%)
3	1.2 equiv. triphosgene	3.6 equiv. DIPEA	CH_2Cl_2	r.t.	6 d	1/4 (15%)
4	1.2 equiv. triphosgene	3.6 equiv. DIPEA	CH_2Cl_2	Δ	4 d	1/4
5	1.5 equiv. triphosgene	4.5 equiv. DIPEA	CH_2Cl_2	r.t.	2 d	1/4 (22%)
6	1.2 equiv. triphosgene	7.2 equiv. DIPEA	CH_2Cl_2	r.t.	2 d	No conversion
7	1.8 equiv. diphosgene	3.6 equiv. DIPEA	CH_2Cl_2	r.t.	1 d	Full conversion (48%)
8	1.8 equiv. diphosgene	3.6 equiv. Et_3N	CH_2Cl_2	r.t.	2 d	Complex reaction mixture
9	1.8 equiv. diphosgene	3.6 equiv. DBU	CH_2Cl_2	r.t.	2 d	Complex reaction mixture
10	3 equiv. diphosgene	6 equiv. imidazole	CH_2Cl_2	r.t.	3 d	Complex reaction mixture
11	3 equiv. diphosgene	6 equiv. K_2CO_3	CH_2Cl_2	r.t.	4 d	1/10
12	3 equiv. diphosgene	6 equiv. DIPEA	THF	r.t.	19 h	Complex reaction mixture
13	3 equiv. diphosgene	6 equiv. DIPEA	ACN	r.t.	1 d	Complex reaction mixture
14	1.2 equiv. $POCl_3$	3.6 equiv. DIPEA	CH_2Cl_2	r.t.	4 d	No conversion
15	1.2 equiv. triphosgene	-	CH_2Cl_2	r.t.	1 d	No conversion
				Δ	4 d	1/1
16	3 equiv. diphosgene	-	CH_2Cl_2	Δ	21 h	Full conversion (50%)

[a] Based on HPLC-MS, isolated yield between parentheses.

Diphosgene was evaluated as an alternative source of phosgene and gave full conversion (entry 7). Nevertheless, a significant amount of side product, formed by reaction between DIPEA and excess tri- or diphosgene, impeded the purification which resulted in low yields. This side product was identified as 1,3-diethyl-1,3-diisopropylurea, formation of which was confirmed by mixing DIPEA with

diphosgene (*see* Experimental Procedures).[208] A related reaction of Et_3N and phosgene mentions the formation of intermediate 1,1'-carbonylbis(triethylammonium)chloride. The urea can lead to acylation of an NH in the diketopiperazine starting material **2b** or desired product **237b** generating more side products and complicating the reaction mixture.[209] This also helps explain why other trialkylamine bases still resulted in complex mixtures consisting of more than 5 compounds, which could not be identified as starting material **2b** or desired product **237b**, as analogous urea side products can be formed (entries 8-10). Only K_2CO_3 gave satisfactory results (entry 11). The use of other solvents than dichloromethane also resulted in complex reaction mixtures consisting of several compounds, which could not be identified as starting material **2b** or desired product **237b** (entries 12-13).

Phosphoryl chloride ($POCl_3$), known to react with amides to form imidoyl chlorides and therefore used in Vilsmeier-Haack reactions,[210-211] was examined as an alternative electrophile under similar reaction conditions. Its use did not result in an analogous reaction (entry 14).

The reaction was also run in the absence of base. Considering the proposed reaction mechanism no base is needed. When the substrate was stirred at room temperature in the presence of triphosgene, no conversion was detected. Under reflux conditions, the reaction proceeded partly (entry 15) and the formation of the urea was avoided. Finally, full conversion was achieved by using an excess of diphosgene (entry 16). Under these conditions, α-chloroamine **237b** was obtained as the major product in the crude mixture. Isolation by column chromatography lowered the final yield due to the polarity of the compounds, which were largely retained on the column. Several solvent mixtures for normal- (petroleumether/EtOAc (+Et_3N)) and reversed-phase (H_2O/ACN) chromatography were examined, but the purification still resulted in major product losses.

The reaction proved successful for all four stereoisomers of cyclo(Trp, Pro) **2**. Table 30 shows the isolated yields of the resulting pentacycles **237a**-**d**.

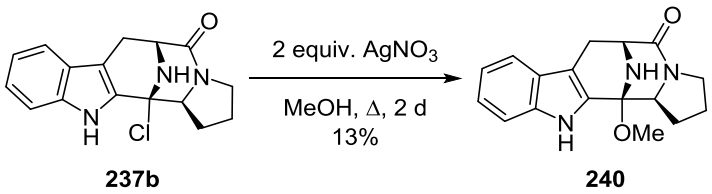
Table 30: Yields for the isomers of α-chloroamine 237.

237	Starting compound	Y (2-3 steps)
a	Cyclo(L-Trp, L-Pro)	40%
b	Cyclo(D-Trp, L-Pro)	50%
c	Cyclo(L-Trp, D-Pro)	24%
d	Cyclo(D-Trp, D-Pro)	46%

2.3.5. Derivatization of the α-chloroamine

Since *trans*-diastereomer **237b** gave the best isolated yield, it was chosen as a substrate to develop a small library of compounds. In research on the cyclo(Trp, Pro) natural products, typically the *cis* configuration, originating from the natural L-amino acids, is studied. However, compounds containing unnatural D-amino acids also possess biological activity. Tadalafil **20** is an example of a commercially available drug used for the treatment of erectile dysfunction, which contains a D-tryptophan unit (Figure 6).[34]

The most obvious position to try to derivatize structure **237b** was at the chlorine atom. The goal was to replace the chlorine atom with various groups. Neutral nucleophiles were evaluated first. Dissolving **237b** in methanol or water or stirring with 3 equivalents of allylamine gave no conversion of the starting material. To stimulate the substitution of chlorine with a methoxy group (**240**), silver nitrate was added as a Lewis acid to a solution of **237b** in methanol. Precipitation of the formed silver chloride salts should drive the reaction to completion. After a week at room temperature, no reaction was detected, but two days of reflux engendered complete conversion (Scheme 52). The silver salts proved to be tedious to remove, even after several filtrations. Other Lewis acid catalysts can solve this problem. However, an alternative method was simultaneously being investigated and provided the desired substitution. Therefore, this method was not pursued any further within the timeframe of this thesis.



237b **240**

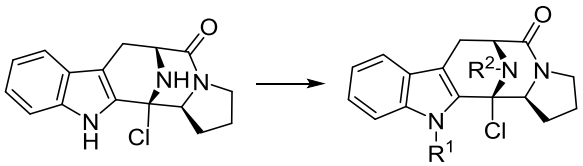
Scheme 52: Initial derivatization of 237b.

Some cyclic compounds that also contained an α-chloroamine are known to exhibit a remarkable reactivity towards nucleophiles under basic conditions.[212-218] In view of such exploration, a short screening of several protecting groups was performed (Table 31). Indeed, since there are two secondary amines present in **237b**, the possibility exists that the nucleophile would merely react as a base if no protecting group was present.

Several protecting groups were introduced. The reactions were followed up using HPLC-MS. The introduction of the protecting group on **237b** was confirmed by the molecular mass. Tentative assignments for **242** and **246** are based on the ^{1}H NMR spectra of the crudes showing the strong decrease in integration of the indole NH signal in comparison with the integration of the other indole

signals. In the case of **245**, two indole NH signals were present, one corresponding to the starting material and one to the acylated product. The sum of the integration of these two signals corresponded to the integration of CH-4 or CH-7 on the indole and the retention of the indole NH was assigned. From this screening of different protecting groups, it became apparent that the indole NH generally reacts more easily with electrophiles and is protected first (entries 1, 2, 4, 7), except in the case of acetyl chloride (entry 5). It is rather counter-intuitive that the secondary amine is less nucleophilic than the indole amine function, which will probably be effectuated by the nearby chlorine atom. For the introduction of the methyl groups extra portions of reagents were added until complete conversion was obtained (entry 8). The first methylation, of the indole nitrogen, occurred very smoothly (entry 7). The introduction of a second methyl group, on the bridge nitrogen, required longer time and more reagents (entry 8). Hence, both selective protection of the indole nitrogen (generally) or the bridge nitrogen (acetyl) is possible.

Table 31: Screening the conditions for the introduction of protecting groups on compound 237b.



237b **Cpd**

Entry	Cpd	R =	Reaction conditions	R^1	R^2	Result[a]
1	**241**	Boc	3 equiv. Boc_2O, 0.2 equiv. DMAP ACN, r.t., 3 h	Boc	H	Complete conversion (98%)
2	**242**	MEM	5 equiv. MEMCl, 6 equiv. DIPEA CH_2Cl_2/THF, r.t., 2 d	MEM	H	Complete conversion (not isolated)
3	**243**	Cbz	2.5 equiv. CbzCl, 2.5 equiv. NaH DMF, r.t., 1 d	-	-	No conversion
4	**244**	Bn	2.5 equiv. BnBr, 2.5 equiv. NaH DMF, r.t., 1 d	Bn	H	Complete conversion (56%)
5	**245**	Ac	2.2 equiv. AcCl, 2.2 equiv. Et_3N CH_2Cl_2, r.t., 2 h	H	Ac	Conversion 50% (not isolated)
6		Me	3.5 equiv. MeI, 3 equiv. Ag_2O Acetone, Δ, 20 h	-	-	Complex reaction mixture

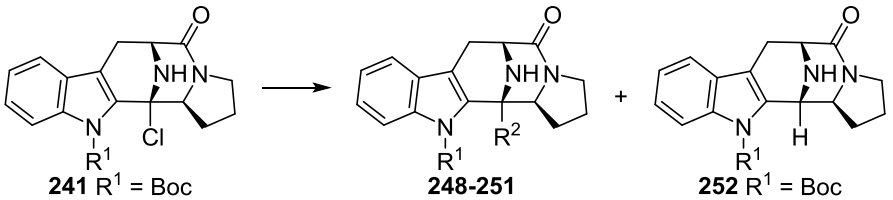
7	**246**	Me	2.5 equiv. MeI, 2.5 equiv. NaH ACN, r.t., 1 h	Me	H	Complete conversion (not isolated)
8	**247**	Me	3.5 equiv. MeI, 3.5 equiv. NaH DMF, r.t., 1 d	Me	Me	Complete conversion (48%)

[a] Conversion obtained by integration of the 220 nm UV-signals in the HPLC chromatogram. Isolated yield between parentheses.

When examining the substitution of the chlorine atom with basic nucleophiles, protecting groups were introduced on the secondary amines to prevent the basic nucleophiles from acting as a base. However, the introduction of such a protecting group was generally only accomplished on the indole nitrogen.

Consequently, in a next step, some of the monoprotected compounds were reacted with a basic nucleophile. Grignard reagents were used (Table 32). In all cases, an excess amount of nucleophile was used to compensate for any loss of nucleophile by taking up a proton from the bridge amine NH and complete conversion was detected with HPLC-MS analysis (entries 1-3). In the case of entries 2 and 3, HPLC-MS analysis indicated the presence of another product in the reaction mixture. According to the mass spectrum, this product (**252**) had lost the chlorine atom on the bridgehead. The introduction of a hydrogen atom replacing chlorine can be explained by a magnesium transfer reaction from the preformed Grignard reagent to the α-chloroamine, which is followed by protonation of the magnesium intermediate by residual water or another proton source. This implies the presence of a Grignard reagent derived from a magnesium-halogen exchange in the α-chloroamine. However, the experiment to use compound **241** itself as the starting material for the formation of a Grignard reagent failed (entry 4). No Grignard reagent was formed as the magnesium remained unaltered after refluxing the suspension and to confirm this observation, allyl bromide was added, which indeed gave no allylated product.

Table 32: Replacing the chlorine atom in monoprotected 237b derivatives using Grignard reagents.



Entry	R^1	Reaction conditions	Anticipated product	Result[a],[b]
1	Bn	5 equiv. BnMgBr CH_2Cl_2, r.t., 3 h	**248** R^1 = Bn, R^2 = Bn	Complete conversion to **248**
2	Boc	2 equiv. vinylmagnesium bromide THF, r.t., 1.5 h	**249** R^1 = Boc, R^2 = $CHCH_2$	Complete conversion mixture of **249** and **252**
3	Boc	5 equiv. BnMgBr CH_2Cl_2, r.t., 2 h	**250** R^1 = Boc, R^2 = Bn	Complete conversion mixture of **250** and **252**
4	Boc	1.05 equiv. Mg, 1.5 equiv. allyl bromide THF, r.t., 1.5 h – Δ, 1.5 h	**251** R^1 = Boc, R^2 = $CH_2CH{=}CH_2$	No conversion

[a] Conversion was determined by integration of the MS-signals in the HPLC-MS chromatogram.
[b] Compounds were not isolated.

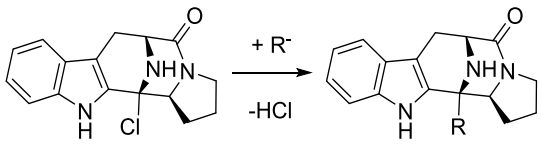
Until now, protecting groups were introduced to prevent the loss of the added nucleophile. However, compound **237b** as such was also reacted with several O-, N-, S- and C-nucleophiles without the use of protecting groups (Table 33). Since no protective groups were used, three equivalents of nucleophile were added. However, good conversion was also achieved using only 1.5 equivalents of nucleophile in particular cases (**255** , **257** and **260**). Generally these reactions proceeded smoothly. Therefore, the introduction of protecting groups on the pentacyclic scaffold prior to the substititution of chlorine with basic nucleophiles was omitted as an unnecessary step.

Unfortunately, separation of the desired products from excess reagent and/or remaining substrate proved tedious due to the polar nature of the materials. Therefore, when the compounds were not immediately obtained in pure form, they were only recovered in low yields by pTLC. Poor solubility of some derivatives was used to isolate these compounds by decanting the solvent from the insoluble pure product. For that reason the more bulky nucleophile 1-naphthylmethylamine was added to the series of derivatives. The naphthyl group may lower the solubility, however decantation also resulted in a low isolated yield of **261**. In some cases the purification failed and the pure product could not be retrieved (e.g. **266** or **268**). Eventually, it was found that these type of compounds could also be

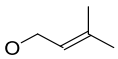
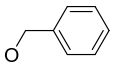
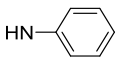
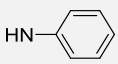
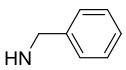
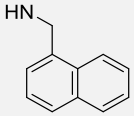
purified with reversed-phase chromatography, but the isolated yields remained low (e.g. **270** and **274**).

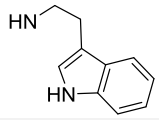
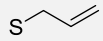
The use of sodium hydride to activate the nucleophiles was necessary since reaction did occur with allylamine when treated with sodium hydride, while it did not proceed with allylamine alone. Some of the reactions with nucleophiles such as cyanide or azide failed to proceed under the current conditions. Compounds as **253**, **263** or **271** are appealing, since the introduced functionalities offer the possibility for further modification.

Table 33: Derivatives of 237b.



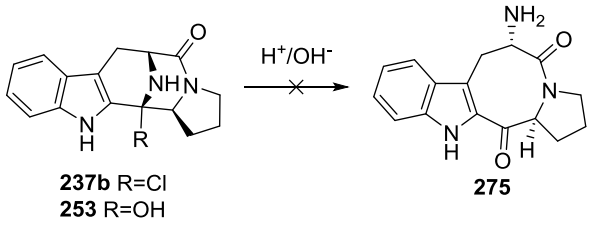
237b **Cpd**

Cpd	R	Reaction conditions	Yield
253	OH	2 M NaOH H_2O, Δ, 2 h	65%
240	OMe	4 M NaOMe MeOH, Δ, 2.5 h	96%
254		3 equiv. allyl alcohol + 3 equiv. NaH THF, r.t., 1 h	86%
255		1.5 equiv. prenyl alcohol + 1.5 equiv. NaH THF, r.t., 1 h	33%[c]
256		3 equiv. phenol + 3 equiv. NaH THF, r.t., 3 d	19%[b]
257		1.5 equiv. benzyl alcohol + 1.5 equiv. NaH THF, r.t., 40 min	26%[a]
258		3 equiv. allylamine + 3 equiv. NaH THF, r.t., 30 min	77%
259		1 equiv. aniline + 2 equiv. KO*t*Bu THF, r.t., 1 h	2%[a]
259		3 equiv. aniline + 3 equiv. NaH THF, r.t., 1 h	24%[b]
260		1.5 equiv. benzylamine + 1.5 equiv. NaH THF, r.t., 1 h	28%[a]
261		3 equiv. amine + 3 equiv. NaH THF, r.t., 3 h	36%[c]

262		3 equiv. tryptamine + 3 equiv. NaH THF, r.t., 4 h	31%[c]
263	NH_2	1.5 equiv. $NaNH_2$ THF, r.t., 2 h	(Incomplete conversion) -
264	N_3	1.5 equiv. NaN_3 THF, r.t., 4 d	No conversion
265		3 equiv. allyl mercaptan + 3 equiv. NaH THF, r.t., 3 d	32%[a]
266	Me	1.5 equiv. MeLi THF, -78 °C , 1 h	(Complete conversion) Not isolated
267	Bu	3 equiv. *n*BuLi THF, -78 °C – r.t., 30 min	12%[a]
268	*s*Bu	3 equiv. *s*BuLi THF, -78 °C , 1 h	(Complete conversion) Not isolated
269	*t*Bu	3 equiv. *t*BuLi THF, -78 °C , 2 h	No reaction
270	Ph	2 equiv. PhLi THF, -78 °C, 3 h	(Complete conversion) 12%[c]
271	CN	1.2 equiv. KCN DMSO, r.t., 11 d	No reaction
271	CN	1.2 equiv. KCN DMSO, 60 °C, 1 h	No reaction
271	CN	3 equiv. ACH +3 equiv. Et_3N THF, 100 °C, 1 d	No reaction
272	CH_2CN	3 equiv. ACN +3 equiv. LDA 0 °C, 4 h	mixture of polar unseparable compounds
273	Bn	1.5 equiv. BnMgBr THF, r.t., 5 h	(trace of product) -
274	$CHCH_2$	3 equiv. vinylmagnesium bromide THF, r.t., 1 h	(Complete conversion) 27%[c]

[a] Isolated yield after purification by pTLC.
[b] Isolated yield after purification by decantation.
[c] Isolated yield after purification by reversed-phase chromatography.

In a 'typical' Vilsmeier-Haack reaction the α-chloroamines are unstable intermediates and undergo hydrolysis to the final product. In this case the newly formed product **237** did not readily hydrolyze to give the eight-membered ketone ring system. Hydrolysis of the bridged structure **237b** or **253** under acidic conditions proved unsuccessful with full recovery of the starting material (Scheme 53). Using sodium hydroxide, compound **253** was isolated (Table 33). The particular stability of the α-chloroamine can be attributed to its bridged structure.



Scheme 53: Ring opening reaction.

2.3.6. Biological testing

The reported structures are the first examples of a new class of brevianamide F analogues, bearing the 3,5-bridged piperazin-2-one core. A preliminary evaluation of the bioactivity of these interesting materials was conducted.

The antimicrobial activity of compounds **237b** and **240** was tested by BCCM^{TM}/LMG (Laboratory of Microbiology, Ugent) against a panel of four bacterial strains: the Gram-negative *Escherichia coli* LMG 8063 and *Klebsiella pneumonia* LMG 2095, and the Gram-positive *Staphylococcus aureus* LMG 8064 and *Bacillus substilis* LMG 13579. No antimicrobial effect was observed based on visual assessment of turbidity caused by bacterial growth.

A subset of compounds was tested against different targets that were chosen based on the interest in the laboratory for those targets[219-220] and on the basis of biological activities displayed by natural product analogues of these compounds (Table 34).

Table 34: Screening of different targets. The values express the percentage inhibition (of the control).

		237b		254		255		257		20	
a	**α2 (non-selective)[a],[b]**	60 nM	6 µM	60 nM	6 µM			60 nM	6 µM		
		-7.1	-5.5	-8.4	-13.1			-9.6	-7.0		
b	**D1[a],[c]**	2.5 µM	25 µM			2.5 µM	25 µM	2.5 µM	25 µM		
		-6	2			3	-6	2	3		
c	**N neuronal α7[a],[d]**	7 µM	70 µM			7 µM	70 µM	6.1 µM	61 µM		
		2	-15			-8	-7	-8	-4		
d	**N muscle-type[a],[e]**	20 µM	0.2 nM			20 µM	0.2 nM	17 µM	0.17 nM		
		5	7			-6	-3	0	-5		
e	**Serotonin 5-HT1 (non-selective)[f]**	11 µM	0.11 nM			11 µM	0.11 nM	9.5 µM	95 µM		
		0	3			11	8	-4	-3		
f	**PDE5(h) (non-selective)[g]**	0.7 µM	70 µM	0.7 µM	70 µM			0.7 µM	70 µM	0.7 µM	70 µM
		-1.0	7.2	0.0	16.8			0.9	18.7	101.8	100.9
g	**Tubulin polymerization[h]**	12 nM	0.12 mM			12 nM	0.12 mM	12 nM	0.12 mM		
		-8	-14			-10	-8	-14	-10		
h	**BCRP (h) inhibition[i]**	5 µM	50 µM			5 µM	50 µM	4.3 µM	43 µM		
		0.1	10.8			21.5	**46.5**	6.9	**40.6**		

All assays were run by Cerep, France. For more details, *see* Experimental procedures. All values are the mean of two replicates. The test concentrations that were used, are based on the IC_{50} values of the reference compounds and the hundredfold or tenfold thereof. [a] Antagonist radioligand. [b] Reference: yohimbine (IC_{50} = 58.7 nM). [c] Reference: SCH 23390 (IC_{50} = 0.242 nM). [d] Reference: α-bungarotoxin (IC_{50} =0.7 nM). [e] Reference: α-bungarotoxin (IC_{50} =2 nM). [f] Reference: serotonin (5-HT) (IC_{50} = 0.0011 µM). [g] Reference: dipyridamole (IC_{50} = 0.7 µM). [h] Reference: vinblastine (IC_{50} = 1200 nM). [i] Reference: KO143 (IC_{50} = 480 nM).

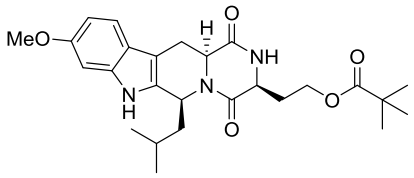
The α-chloroamine **237b** and two derivatives **254** and **257** were submitted to a competitive binding assay against the α2 receptor, an adrenergic receptor localized in the central nervous system (Table 34, entry a). The α2-receptor was chosen since the scouting of the chemical space for the closest resemblance of the newly reported compounds pointed towards yohimbine, which contains a tryptamine unit enclosed in a pentacyclic structure. This molecule was used as a reference compound in the binding assay. Values are expressed as the decrease of radioligand specific binding in the presence of the compounds and were determined by scintillation counting.[221] As can be seen from Table 3, no significant binding to the α2-receptor could be detected for the selected compounds.

α-Chloroamine **237b** and two derivatives **255** and **257** were also tested for binding against a set of receptors which were of interest to the laboratory: the D1 dopamine receptor which is found in the central nervous system (Table 34, entry b), the α7 nicotinic receptor and muscle-type nicotinic receptor which are both a type of nicotinic acetyl choline receptors (Table 34, entries c and d), and the 5-HT1 serotonin or 5-hydroxytryptamine receptor (Table 34, entry e). These receptors influence various biological and neurological processes. No significant binding to these receptors could be detected.

The compounds were also tested for their inhibitory activity against the phosphodiesterase type 5 (PDE5) enzyme, which plays an important role in the cardiovascular system. Tadalafil **20** is a potent inhibitor of these enzymes, and bears structural resemblance to the tested compounds.[222] Cyclic guanosine monophosphate (cGMP) is broken down by the enzyme into guanosine-5'-triphosphate (GMP). The decrease in conversion of radiolabeled cGMP to GMP in the presence of the compounds is reflected in the percentage inhibition measured by scintillation counting.[223] Unfortunately, α-chloroamine **237b** and derivatives **254** and **257** exhibit a very low potency for PDE5 inhibition (Table 34, entry f). The best result was obtained for **257**, containing an aromatic benzyl sidechain. Of the tested compounds **257** indeed displays the most resemblance with the 1,3-benzodioxole-bearing tadalafil **20**.

The fungal metabolite tryprostatin A **6** was identified as an inhibitor of tubulin polymerization and thus prevents cell cycle progression at the M-phase.[224] Compounds **237b**, **255** and **257** do not impair the microtubule assembly at the tested concentrations (Table 34, entry g).

Several diketopiperazines, including fumitremorgin C **7** and analogues reverse multidrug resistance in cells transfected with the breast cancer resistance protein (BCRP).[17-18, 109] The BCRP is a transmembrane transporter that contributes to the resistance of cancer cells to chemotherapeutic agents such as mitoxantrone, topotecan and methotrexate, by removing these substances from the cell. Interestingly, compounds **255** and **257** display a significant inhibition of BCRP (46.5% and 40.6% at 50 and 43 µM, respectively). The presence of a more bulky sidechain replacing the chlorine atom is required for activity, since **237b** does not display a significant degree of inhibition. The IC_{50}'s of **255** and **257** are around a hundred fold of the reference compound Ko 143 (**276**), also a diketopiperazine (Figure 30).



276

Figure 30: Ko 143 (276).

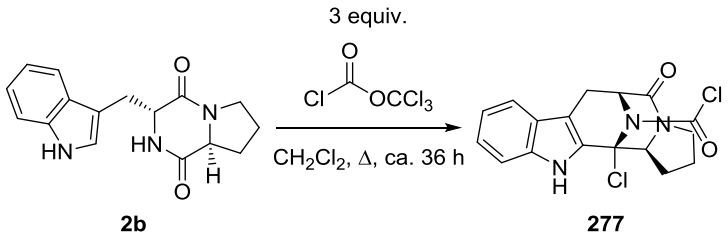
2.3.7. Carbamate and urea derivatives of the α-chloroamine

The reaction conditions using an excess of diphosgene provided the α-chloroamine **237** as the major product in the crude mixture but some other (side) products were also formed. Based on HPLC-MS analysis during the reaction, it was found that one of these other peaks displayed a mass corresponding to the α-chloroamine **237** with an extra methoxycarbonyl group attached to it. Since the samples were dissolved in MeOH, this solvent was most probably responsible for this observation. During flash chromatography, sometimes a small fraction was collected that was not the expected product **237**, and that did not resemble the starting material. Analysis with HPLC-MS and NMR spectroscopy again demonstrated the addition of the –COOMe unit to structure **237**. From time to time some methanol was added to dissolve the crude product to coat it on silica for column chromatography. Thus, the methoxy group was derived from a reaction of methanol with a derivative of **237**. Since methanol apparently reacted very easily with this unknown compound, the solvent used for the HPLC-MS samples was switched to acetonitrile.

When the reaction of **2b** with diphosgene was left to stir for a longer period of time the fraction of α-chloroamine **237** started to decline, as it was converted into another product. The HPLC-MS analysis revealed that the newly formed compound contained two chlorine atoms.

We propose that the excess diphosgene that is added to the reaction, reacts with the bridging secondary amine to yield a carbamoyl chloride **277** (Scheme 54). Upon exposure to methanol, the methyl carbamate **278** is immediately formed. Nonetheless, the carbamoyl chloride **277** is rather stable, since the work-up involves water. It is curious that the carbamoyl chloride **277** does not readily react with water forming the unstable carbamic acid, which would result again in **237b**, despite the swift reaction with methanol.

The reaction of cyclo(D-Trp, L-Pro) **2b** with diphosgene was stirred until all material was cleanly converted to the carbamoyl chloride **277**. The product that was obtained was already quite pure (±90%) and the crude yield was high (90%). The newly formed carbamoyl chloride **277** offers an alternative site of modification and enables the synthesis of novel derivatives.

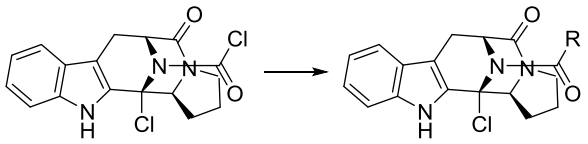


2b **277**

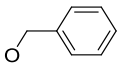
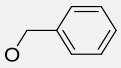
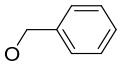
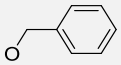
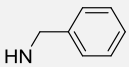
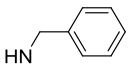
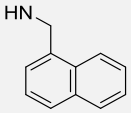
Scheme 54: Reaction of DKP 2b with diphosgene leading to carbamoyl chloride 277.

To synthesize new carbamate or urea derivatives, several alcohols and amines were added to the carbamoyl chloride **277** (Table 35). Partial degradation of **277** during the reaction with alcohols and amines led to mixtures of the targeted carbamate compound and compound **237b**.

Table 35: Carbamate and urea derivatives of carbamoyl chloride 277.



277 **Cpd**

Entry	Cpd	R	Reaction conditions	Yield
1	**278**	OMe	MeOH, r.t.	65%
2	**279**		2 equiv. benzyl alcohol, 2 equiv. Et_3N THF, r.t., 12 d	33%[a]
3	**279**		1.5 equiv. benzyl alcohol, 1 equiv. Et_3N THF, Δ, 12 d	29%[a]
4	**279**		1.5 equiv. benzyl alcohol, 1 equiv. Et_3N Neat, r.t., 2 h	8%[a]
5	**279**		3 equiv. benzyl alcohol Neat, r.t., 4 d	36%[a]
6	**280**		3 equiv. allyl alcohol Neat, r.t., 4 d	44%[b]
7	**281**		1.3 equiv. benzylamine, 1.3 equiv. K_2CO_3 THF/H_2O, r.t., 4 h	38%[a]
8	**281**		3 equiv. benzylamine Neat, r.t., 2 h	58%[b]
9	**282**		3 equiv. 1-naphthylmethylamine Neat, r.t., 4 d	32%[c]
10	**283**		3 equiv. *tert*-butanol Neat, 30 °C, 4 d	no conversion

[a] Isolated yield after purification by reversed-phase chromatography.
[b] Isolated yield after purification by normal-phase chromatography.
[c] Isolated yield after purification by decantation.

A base such as triethylamine or K_2CO_3 was added to neutralize the HCl released during the reaction (entries 2-4, 6) and tetrahydrofuran was used as a solvent, since the starting material did not (re)dissolve in dichloromethane, acetonitrile or ethyl acetate. The use of a solvent slowed down the reaction tremendously. Both the addition of base and solvent ultimately proved unnecessary as the reactions proceeded well under neat conditions (entries 1, 5, 6, 8, 9). As a result, only liquid alcohols or amines were screened and an excess amount was used to allow the reaction to stir. In the case of compound **281** and **282**, the viscous mixture precipitated shortly after the addition of the amine, showing that the amine acted as a base and formed an ammonium chloride precipitate. The reaction with *tert*-butanol was unsuccessful (entry 10). Despite the easy reaction of the carbamoyl chloride with amines, no dimeric products were observed during the synthesis of the carbamoyl chloride **277** from reaction with amine **237b**.

The reaction of carbamoyl chloride **277** with benzyl alcohol was also performed using continuous flow technology. Syringe pumps were used to supply a flow of reagents to the tube reactor. The main advantages for using meso flow reactors versus batch are the better surface to product volume ratios, which improves heat transfer capabilities and better mixing efficiencies.[225] One syringe pump contained a 0.5 M solution of carbamoyl chloride **277** in dry THF. The second syringe pump was filled with benzyl alcohol. The tube reactor had a volume of 0.3 mL. Depending on the desired residence time and ratio of reagents the flow rates were calculated.

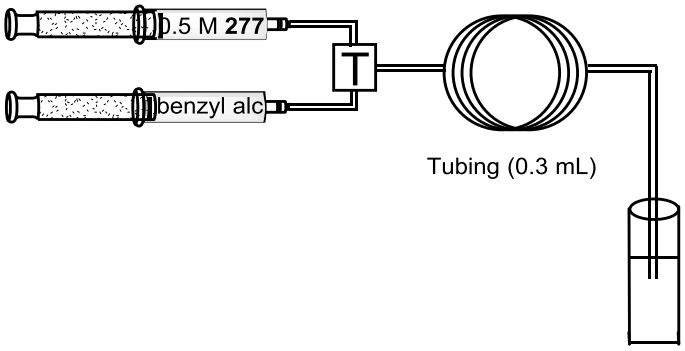


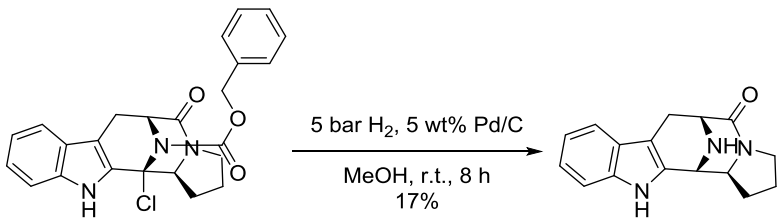
Figure 31: Setup tube reactor with syringe pumps. Tube reactor with internal diameter of 0.508 mm.

The solution of carbamoyl chloride was mixed at the T piece with an excess of benzyl alcohol at 60 °C (Table 36, entry 1). After a residence time of 5 minutes no conversion had taken place and the starting material remained. The temperature was elevated to 80 °C but still no product was obtained (entry 2). Finally, a large excess of benzyl alcohol was used and a trace of the carbamate **279** could be found (entry 3). Again the use of solvent is detrimental for the reaction. Since the reaction in batch was already successful using only 1.5-3 equivalents of benzyl alcohol, the application of the reaction in the meso reactor was not further pursued.

Table 36: Reaction of carbamoyl chloride 277 with benzyl alcohol.

Entry	**Equiv. benzyl alcohol**	**RT (min)**	**T (°C)**	**Result**
1	1.5	5	60	No conversion
2	1.5	5	80	No conversion
3	10	6	80	Trace of **279**

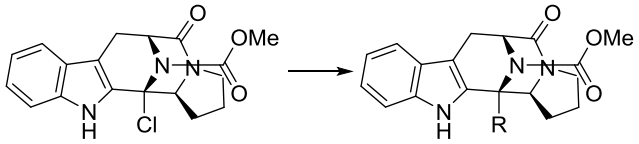
Reaction of the carbamoyl chloride **277** with benzyl alcohol resulted in the synthesis of a benzyloxycarbonyl (Cbz) group (**279**). This moiety can function as a protecting group to selectively shield the bridging amine, which is complementary to the earlier introduction of protecting groups at the indole-N (*see* 2.3.5), and can easily be deprotected by hydrogenolysis (Scheme 55). Hydrogenolysis of **279** also removed the chlorine atom yielding **284** after pHPLC. Analogously, if the reaction with *tert*-butanol would have been successful, a Boc protecting group would have been the result. It was assumed that the shielding of the amine function might facilitate purification by reducing the polarity of the compound. This would eventually lead to derivatives of compound **237b** in a better yield despite the extra steps. However, compound **284** was obtained in low yield as a result of purification, which eliminated this presumed alternative route to derivatives of **237b**.



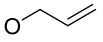
Scheme 55: Hydrogenolysis of the carbamate derivative 279.

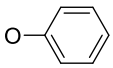
By transforming the carbamoyl chloride **277** to a carbamate or urea, a more stable compound is formed that can undergo further modification. The methyl carbamate **278** was subjected to similar conditions as **237b** to assess the possibility of replacing the chlorine with different nucleophiles. Two compounds were synthesized and prove that this is indeed possible. The starting material **278** was converted to the desired compounds **286** and **287**, next to a side product for which the chlorine atom is replaced with a hydroxyl group (**285**). No loss of the carbamate group was detected, neither were traces found of the product resulting from reaction of the nucleophile with the carbamate moiety.

Table 37: Substitution of the chlorine atom in carbamate derivative 278.



278 **285** R = OH

286 R = 

287 R = 

Cpd	Reaction conditions	Ratio[a]	Yield 286 or 287
286	1.5 equiv. allyl alcohol + 1.5 equiv. NaH THF, r.t., 1 d	**285/286** 3/2	9%[b]
287	1.5 equiv. phenol + 1.5 equiv. NaH THF, r.t., 1 h	**278/285/287** 5/4/11	38%[c]

[a] Ratios obtained by integration of the MS-signals in the HPLC-MS chromatogram.
[b] Isolated yield after purification by reversed-phase chromatography.
[c] Isolated yield after crystallization

One urea **282** and two carbamate **286** and **287** derivatives were also tested for their inhibitory activity against BCRP. No inhibitory activity was observed for any of the tested compounds. This may indicate the necessity of the bridging amine NH for activity (possible hydrogen bonding) or maybe the urea or carbamate groups are too bulky to allow the molecule to interact with the active site.

Table 38: The values express the percentage inhibition of the control.

BCRP (h) inhibition[a]	282	286	287
5 µM	7.1	-5.5	8.2
50 µM	13.3	-0.4	6.0

All assays were run by Cerep, France. For more details, *see* Experimental procedures. All values are the mean of two replicates. The test concentrations that were used, are based on the IC_{50} values of the reference compounds and the hundredfold or tenfold thereof. [a] Reference: KO143 (IC_{50} = 480 nM).

2.3.8. Mechanistic discussion for the substitution of chlorine

The stability of the obtained α-chloroamine **237** originates from its bridged structure and can be explained by the rule of Bredt. The formation of an iminium ion on the bridgehead through elimination of chloride is prevented by the high degree of strain, which would be introduced by the *E*-double bond in a six-membered ring. Nevertheless, this does not correspond to the observed substitution of the chlorine atom.

The α-chloroamine **237b** did not readily react (over a period of 1-3 days) with nucleophiles (methanol, water or allylamine) under neutral conditions (*vide supra*). In contrast, basic nucleophiles reacted smoothly with **237b** at room temperature. Thus, a stronger nucleophile seems to be necessary to mediate the replacement of chlorine. In the case of an E1 or S_N1 type mechanism, the difference in nucleophilicity (e.g. water *vs*. hydroxide) does not have an influence on the reaction rate as the rate-determining step is the loss of chloride and the concomitant formation of a carbocation intermediate.

Literature provides evidence for the participation of nitrogen in the solvolysis of bridgehead chlorides.[212] Possibly, the reactions of **237b** with the nucleophiles go through a strained bridgehead imine or iminium intermediate, thereby exhibiting an anti-Bredt character.[212-218]

These contradictory observations cannot be explained by Bredt's rule and are a proof that Bredt's rule is only an empirical observation and not an absolute rule. A better strategy for assessing the stability of the bridgehead alkene may involve the calculation of olefin strain energy.[226] The olefin strain (OS) corresponds to the difference in energy between the alkene and the corresponding alkane. Calibration using bridgehead olefins of known stability leads to the the definition of three olefin strain ranges: 'isolable', 'observable' or 'unstable'. Through OS calculations, a compound of unknown stability can be allocated to one of these ranges to determine if a putative 'anti-Bredt' compound would be isolable. These calculations are ongoing and will be used to contribute to the discussion on the putative mechanism for the substitution. These results will be disclosed in due time.

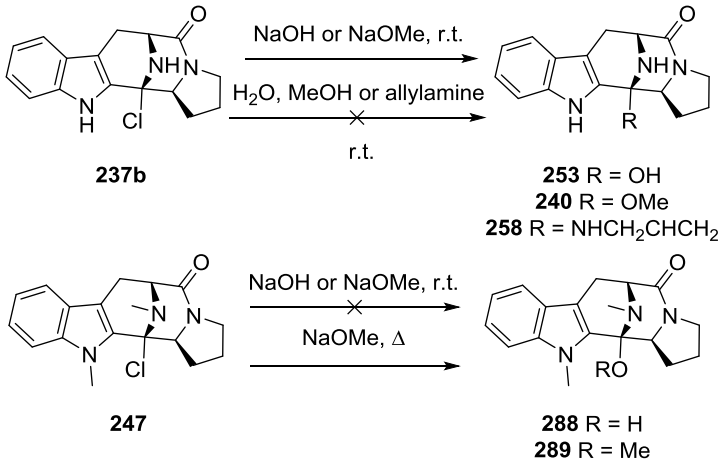
Maybe the basic conditions are required to deprotonate the intermediate iminium species, after expulsion of chloride (in an E1 or S_N1 type mechanism), to the imine and to compensate for the hydrochloric acid that would be released.

A base-promoted elimination like in an E2 type mechanism involving the deprotonation of the bridging secondary amine seems implausible, because no antiperiplanar conformation is possible in

the bicyclic structure. However, this mechanism was supported by an experiment with dimethylated **247**. When compound **247** was treated with sodium methoxide or sodium hydroxide at room temperature no reaction took place, in contrast to the easy conversion of **237b**. If the reaction would proceed via an E1 or S_N1 type of mechanism, the methyl group should not have influenced the outcome. In case of an E2 mechanism, the dimethylated **247** has no proton on the bridging amine, so no elimination can occur and indeed no reaction was observed. When **247** was refluxed in a sodium methoxide solution, substitution was observed, so maybe the S_N1 or E1 mechanism prevailed at elevated temperature.

The reactivity of carbamate **278** with the basic nucleophiles does not concur with the finding that dimethylated **247** does not react with basic nucleophiles at room temperature. Structure **278** resembles the dimethylated structure **247**, as both compounds are substituted at the bridging amine. This rules out the E2 mechanism playing a role for all the observed substitution reactions.

Still, if an E1- or S_N1-type of mechanism is assumed to prevail under these reactions, the presence of the more electron donating methyl group should have facilitated the intermediate imine formation more than the more electron withdrawing ester/carbamate group.



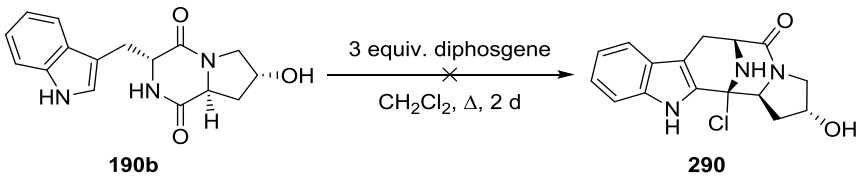
Scheme 56: Observed reactivities of different α-chloroamine derivatives towards nucleophiles.

Assuming that the basic conditions were necessary for quenching the hydrogen chloride released during the reaction, can account for the lack of conversion when only 1.2 equivalents of KCN or 1.5 equivalents of benzylmagnesium bromide were used. In theory, one equivalent would be necessary to neutralize the HCl formed while another equivalent would be necessary to function as a nucleophile. When potassium cyanide or sodium azide get protonated the corresponding acids are gaseous and would evolve from the reaction explaining the lack of reaction. In the case of the O-, N- and S-nucleophiles, reducing the number of equivalents did not pose an issue since the neutral

amines, alcohols or thiol would have been nucleophilic enough to react with an intermediate imine. The neutral counterparts of the carbon nucleophiles, on the other hand, will not react. Reactions with the latter nucleophiles were unsuccessful using only 1.2-1.5 equivalents of nucleophile. However, both quenching and substitution reactions would occur simultaneously and not all HCl would be reacted first. Theoretically a conversion of 60-75% should still have been possible, but was never detected. Thus, no conclusive explanation was found for the differences in reactivity, leaving room for debate.

2.3.9. Introducing the α-chloroamine in cyclo(Trp, Hyp)

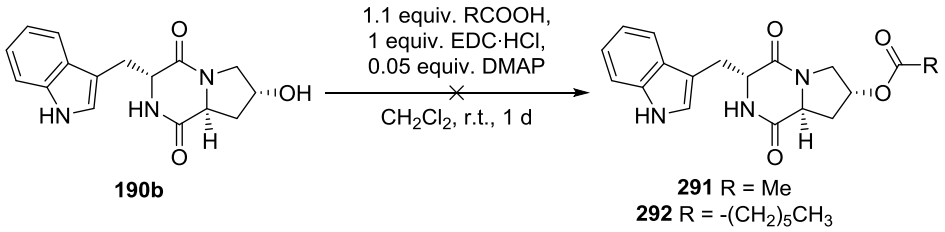
Introduction of the new 3,5-bridge was also investigated for the DKP cyclo(D-Trp, L-Hyp) **190b**. This compound contains a free hydroxyl group, which holds promise for further derivatization studies. Subjecting **190b** to an excess of diphosgene resulted in a complex reaction mixture (Scheme 57). This outcome was to be expected due to the presence of the free hydroxyl group. Introduction of a protecting group or preparation of a derivative that shields the alcohol could help to overcome this problem. Here, the selective introduction of a protecting group on the hydroxyl moiety in the presence of the indole-NH and amide, was the first challenge. According to patent literature, the protecting group, *tert*-butyldiphenylsilyl (TBDPS), has already been selectively introduced on the alcohol in cyclo(D-Trp, L-Hyp) **190b**.[227] However, this group can be removed using HCl in methanol.[228] For the introduction of the α-chloroamine in **190b**, excess diphosgene is used producing HCl. Therefore, the TBDPS group was not evaluated as it would unlikely remain stable under these conditions.



190b **290**

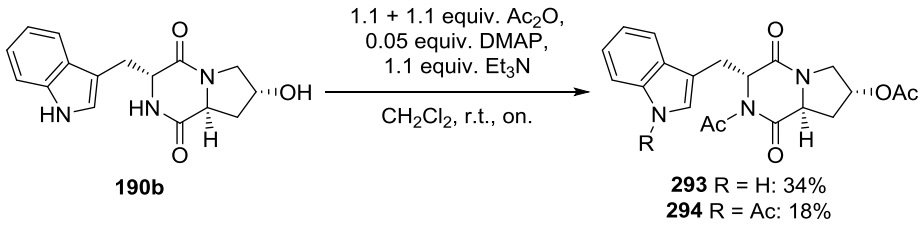
Scheme 57: Unsuccessful synthesis of α-chloroamine 290.

With cyclo(D-Trp, L-Hyp) **190b** already in hand, this compound was used to perform an esterification with acetic acid or heptanoic acid in the presence of EDC·HCl and DMAP. These coupling reactions were unsuccessful due to lack of conversion (Scheme 58).

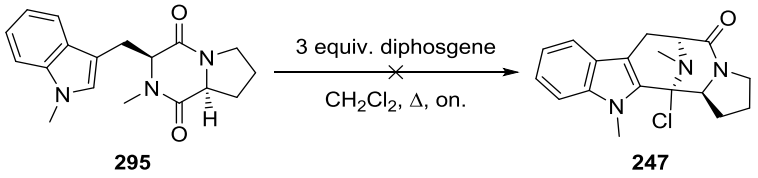


Scheme 58: Unsuccessful coupling of the hydroxyl group in 190b with an acid.

Next, the acetylation of cyclo(D-Trp, L-Hyp) **190b** *via* the addition of acetic anhydride was examined. The reaction was not selective and partial conversion was observed to a di-acetylated DKP **293**. After the addition of an extra equivalent of acetic anhydride full conversion of the starting material **190b** was achieved, but this time the crude mixture was composed of di- and tri-acetylated DKPs **293** and **294** (Scheme 59). Purification with reversed-phase chromatography yielded both compounds **293** and **294**. Spectral analysis revealed that the di-acetylated compound **293** possessed a non-protected indole nitrogen. Since an extra substituent on the amide nitrogen prevents reaction with diphosgene, as confirmed with dimethylated cyclo(L-Trp, L-Pro) **295** (Scheme 60), the di-acetylated **293** could not be used in further steps. The same goes for the tri-acetylated **294**.

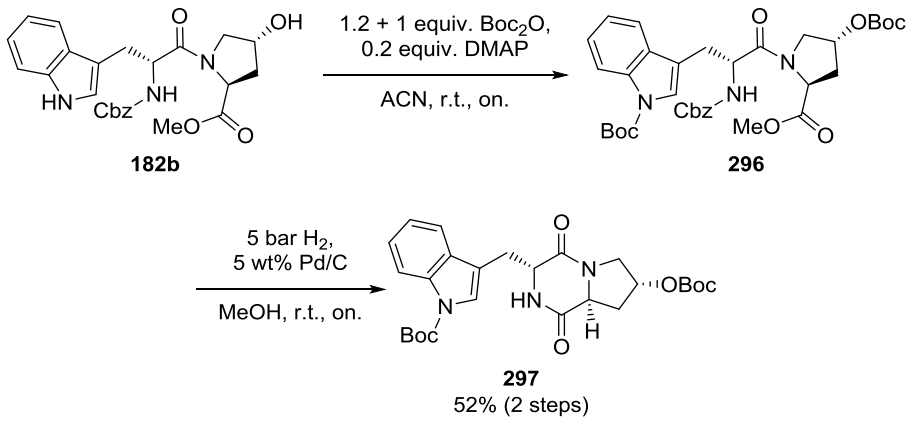


Scheme 59: Acetylation of 190b.



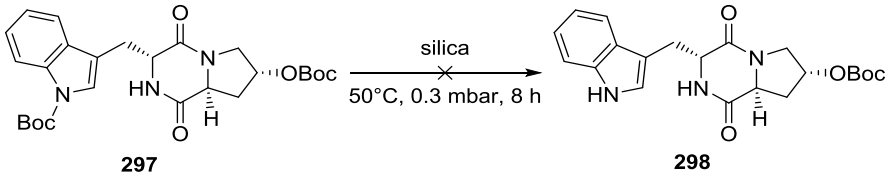
Scheme 60: Methylated DKP 295 is unreactive towards diphosgene.

However, the dipeptide **182b** was also available, which holds the advantage that already one position is protected by the Cbz group. This intermediate **182b** was treated with one equivalent of Boc_2O, but a mixture of mono- and di-Boc-protected dipeptide was obtained. This resulted in incomplete conversion, so an extra equivalent of Boc_2O was added to transform all the material into the di-Boc--protected dipeptide **296** (Scheme 61). Upon deprotection by hydrogenolysis and column chromatography, DKP **297** was isolated in 52% yield over the two steps.



Scheme 61: Synthesis of *N,O*-diprotected 297.

In a next step, selective deprotection of the Boc group on the indole amine from **297** was envisaged, since the presence of the Boc group would reduce the nucleophilicity of the indole. Selective removal of the Boc protective group from nitrogen atoms in conjugation with an aromatic or carbonyl group has been described employing silica gel.[229] However, after heating under reduced pressure for several hours, the starting material was retrieved (Scheme 62).



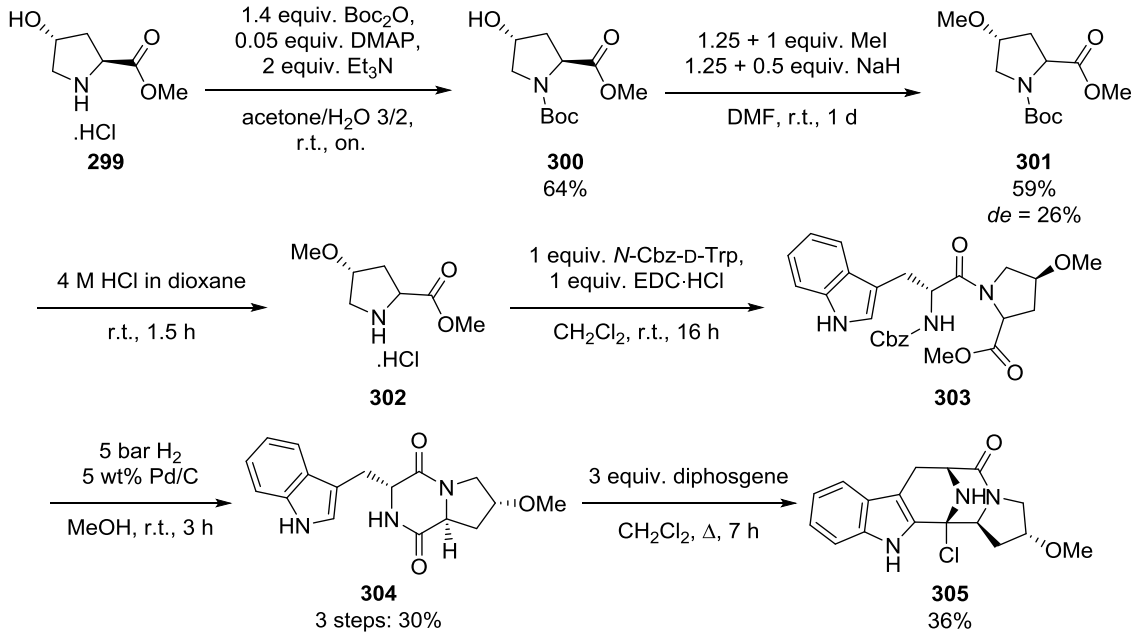
Scheme 62: Unsuccessful selective deprotection of 297.

The attempts to synthesize selectively protected cyclo(D-Trp, L-Hyp) **190b** were unsuccessful so far. Hence, the synthetic route was adapted. The protecting group at the hydroxyl moiety was to be introduced in the starting amino acid, instead of trying to introduce it selectively later on in the synthesis. Starting from the commercially available L-4-hydroxyproline methyl ester hydrochloride **299**, a Boc protecting group was introduced on the amine (**300**, Scheme 63).[230]

Subsequent alkylation of the hydroxyl group was attempted. Reaction with benzyl bromide resulted in a complex reaction mixture. Next, iodomethane was evaluated. Extra portions of iodomethane and sodium hydride were added when the conversion stagnated. The addition of excess sodium hydride caused partial isomerization resulting in diastereomeric **301**.

Next, the Boc protecting group was removed with hydrochloric acid in dioxane.[231] The proline methyl ester **302** was then coupled as such with *N*-Cbz-D-Trp **155b** in the presence of EDC·HCl. The resulting dipeptide **303** was subjected to hydrogenolysis without purification. Spontaneous ring closure of the deprotected dipeptide yielded the diketopiperazine **304**.

The major diastereomer was obtained in 30% yield over the 3 last steps after column chromatography. In the final step DKP **304** was reacted with diphosgene, which yielded its 3,5-bridged counterpart **305** after isolation with pHPLC. No further attempts were made to introduce another protecting group or functionality. Further studies to introduce an easily removable protecting group should make further derivatization at the hydroxyl function accessible.



Scheme 63: Synthesis of α-chloroamine derivative 305.

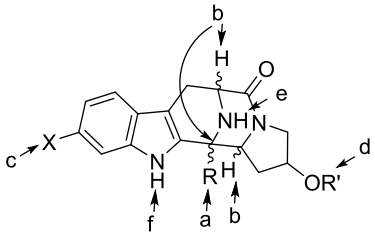
2.3.10. Conclusions

A method for the preparation of 3,5-bridged piperazin-2-ones containing an α-chloroamine functionality from cyclo(Trp, Pro) **2** was presented, by using diphosgene for the formation of the C-C bond affording the pentacyclic scaffold. Density functional theory (DFT) calculations suggest that the α-chloroamine is formed by direct attack by the C-2 atom of the indole group and not by C-3 attack and a subsequent 1,2-shift. The newly obtained bridged structure includes the remarkable feature of a chloro-substituent α to nitrogen, a structural unit which is mostly unstable. Derivatization of the pentacycle by substitution of the chlorine atom offers a new avenue towards synthetic analogues of brevianamides, fumitremorgins and (spiro)tryprostatins. To illustrate this opportunity, a small library of decorated pentacycles was synthesized using a range of O-, N-, S- and C-nucleophiles. A preliminary bioactivity screening of some of the newly developed diketopiperazines revealed significant inhibition of BCRP. Structural modifications to obtain higher BCRP inhibitory potency are possible, as the presence of the α–chloroamine provides an easy way to decorate the novel pentacyclic framework. Besides, other stereoisomers are easily accessible.

In general purifications proved to be extremely cumbersome. To purify the compounds using chromatography a choice had to be made between the lesser of two evils. On the one hand, using normal-phase chromatography the polarity of the compounds causes tailing, resulting in co-elution and loss of the desired compounds. On the other hand, reversed-phase chromatography was well suited to separate the different products, but as the compounds poorly dissolve in acetonitrile or water, major product losses were encountered again. Generally, the successful reactions in this work gave good conversions and improving the purification methods would increase the isolated yields.

IV. Perspectives

Further research towards structural modifications to the 3,5-bridged scaffold in view of obtaining a higher BCRP inhibitory potency are possible, as the presence of the α–chloroamine provides an easy way to decorate the novel pentacyclic framework **360** (Figure 32, modification a). Taking into account the diverse set of nucleophiles that can be used, a wide range of modifications remains to be investigated. Other isomers are also easily accessible (modification b). Alterations to the amino acids parts of the diketopiperazine e.g. substitution of tryptophan (modification c) or functionalization of hydroxyproline (modification d) remain to be investigated, and may reveal compounds with higher potency. The pentacyclic compound **360** also bears multiple nitrogen positions that are easily amenable to further modification (modifications e and f). When the reaction of the α–chloroamine with excess diphosgene proceeds, a carbamoyl chloride is obtained. This functional group enables the introduction of different substituents at the position of the bridging amine (modification e). The carbamate group is a key structural motif in many approved drugs and prodrugs.[232]



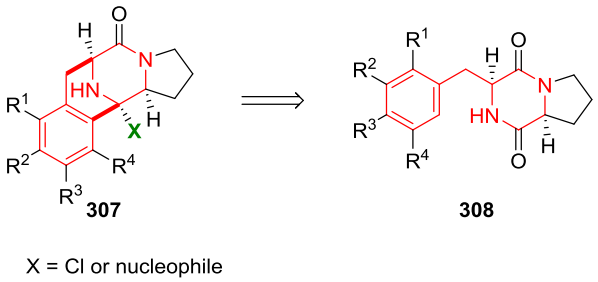
360

Figure 32: Possible sites for modification of the novel 3,5-bridged structure.

When a derivative would be found with improved activity, the multitude of modification sites can be very useful. They can serve as anchoring points for a tracer molecule to investigate the target of the derivative and can give an indication of the parts of the structure that are crucial for its activity.

The use of DKPs as blood-brain barrier shuttle has also been described.[233] So, by introducing well-chosen groups, one can easily modify the lipophilicity of the molecule while attaching the compound to be transported to another part of the molecule.

Moreover, it would be interesting to investigate the substrate scope of the ring forming reaction with diphosgene. On the one hand proline could be replaced by other amino acids to obtain better bioactivities. On the other hand it would be very promising if the method can be applied on diketopiperazines containing an aromatic amino acid other than tryptophan, such as phenylalanine derivatives. Phenylalanine derivatives decorated with various alkoxy or alkylgroups are good nucleophiles and are most likely to react (**308**, Scheme 64). Tetracyclic compounds **307** would be easily accessible in this manner.



Scheme 64: Proposed intramolecular cyclization of phenylalanine-based diketopiperazines.

This could open the way to simplified analogues of other complex natural products such as ecteinascidins (e.g. **309**, Figure 33).[234] Ecteinascidin 743 (**309**) is a marine natural product and a potent antitumor drug. It was recently approved for the treatment of a number of soft tissue sarcomas and ovarian cancer and is known under the commercial name Yondelis® or Trabectedin.[235] Several total syntheses of ecteinascidin 743 (**309**) have been reported, since it cannot be obtained in adequate quantities from natural sources.[236-240] However, these syntheses require many steps and are therefore not suited for scale-up. Ecteinascidin 743 (**309**) is nowadays obtained through a lengthy semi-synthesis (21 steps) from cyanosafracin B **310**, which is readily available through fermentation (Figure 33).[241]

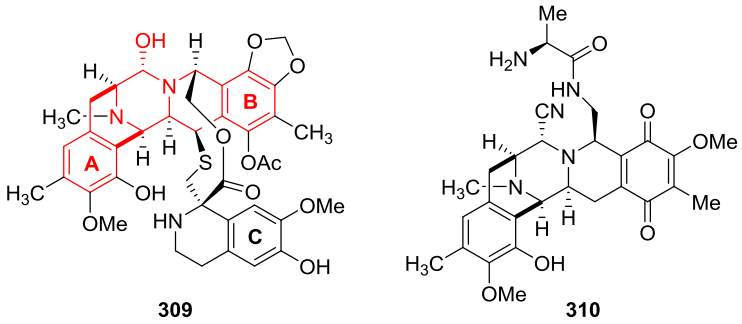
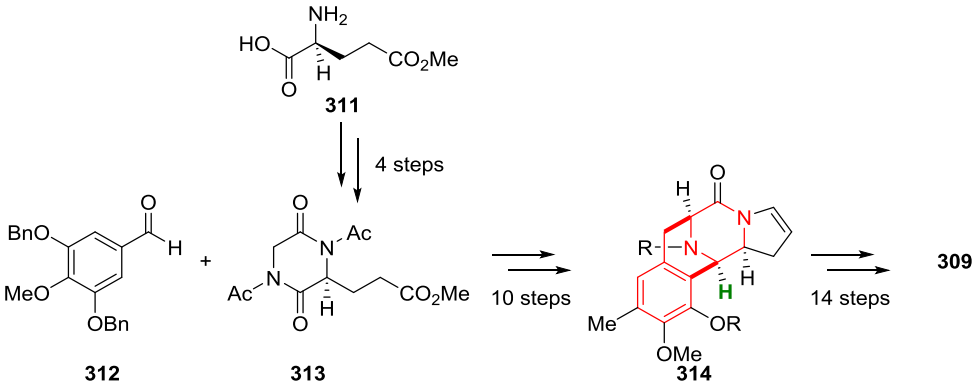


Figure 33: Ecteinascidin 743 (309) and cyanosafracin B 310.

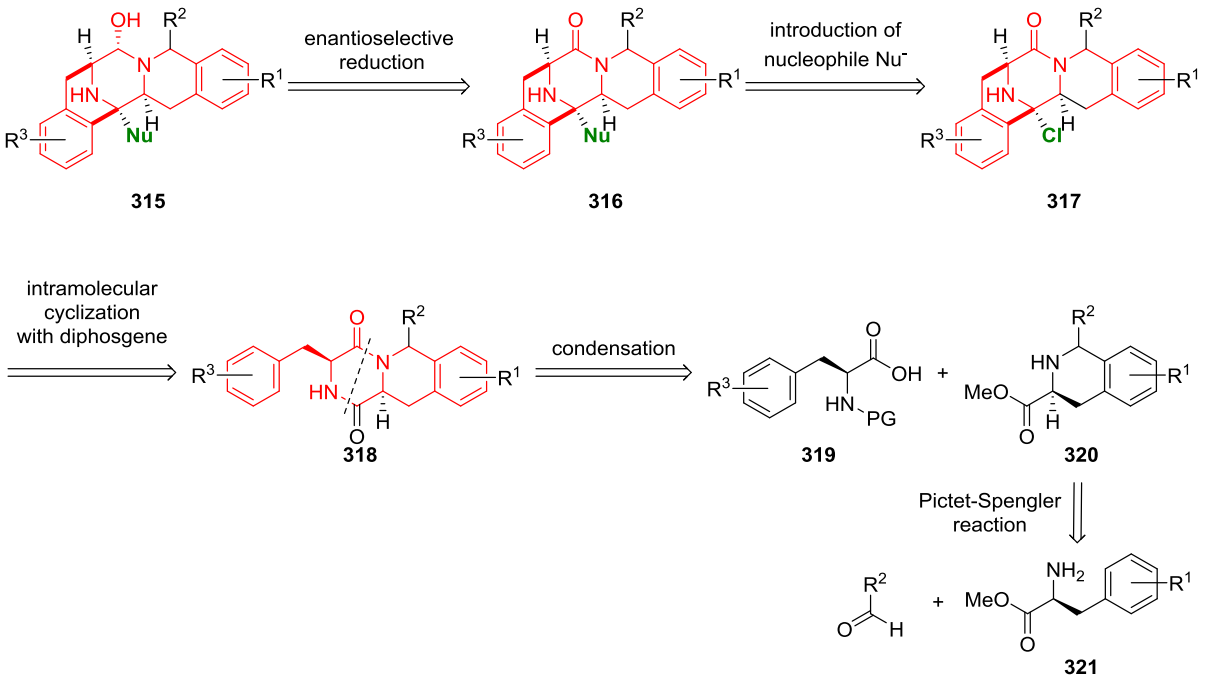
Compounds **307** show much resemblance to one of the key intermediates (**314**) in Fukuyama's recent total synthesis of ecteinascidin 743 (**309**, Scheme 65). The intermediate **314** is synthesized from diketopiperazine **313** in 10 steps. Preparation of **313** itself required 4 steps starting from the natural amino acid, L-glutamic acid derived **311**. From intermediate **314**, the synthesis of Fukuyama describes another 14 steps to obtain ecteinascidin 743 (**309**).[242] The intermediate may be obtained through our reaction in fewer steps.



Scheme 65: Synthesis of ecteinascidin 743 (309) according to the group of Fukuyama.

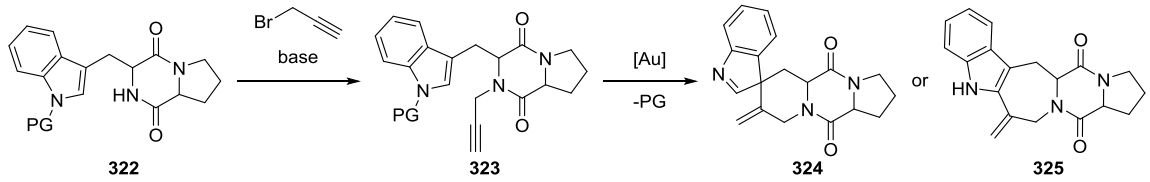
Ecteinascidin 743 (**309**) has a very complex structure and therefore it is not amenable to extensive SAR work. Based on our current methodology, simplified analogues can be proposed with a general structure **315** (Scheme 66). The critical structural features responsible for its activity as antitumor agent are rings A and B and the hemiaminal functionality (highlighted in Figure 33). This framework should be conserved when synthesizing ecteinascidin 743 (**309**) analogues. We therefore propose structure **315**, which may be obtained through our newly discovered diphosgene-induced intramolecular cyclization reaction, as a template for SAR work.

Diketopiperazines **318** can be synthesized from two phenylalanine derivatives **319** and **321**. The amino acid derivative **320** is obtained from **321** *via* a Pictet-Spengler reaction. The reaction of diketopiperazine **318** with diphosgene should provide the pentacyclic α-chloroamine **317**. The chlorine at the bridgehead position of **317** again leaves room for further modifications (**316**). The analogues **315** would result from enantioselective reduction of the amide unit. The two rings A and B and the hemiaminal, necessary for retaining activity, are present.



Scheme 66: Retrosynthetic analysis for the synthesis of simplified ecteinascidin 743 analogues 315.

From the basic scaffold **322** alternative strategies can still be designed to prepare annulated or spiro-derivatives with different ring sizes that are worthwhile to elaborate further such as in Scheme 67. The basic diketopiperazine **322** can, for example, be decorated with a propargyl unit. Subsequent intramolecular cyclization of **323** can be accomplished by means of a gold catalyst. The use of gold(III)chloride to mediate ring formation has been investigated at our department for the synthesis of isoindoles.[243] The resulting product is expected to be either a spiro-derivative **324** or an annulated pentacycle **325** obtained through a 1,2-shift in **324**. These products possess an exocyclic double bond, which is amenable to further modifications *via* electrophilic addition reactions.



Scheme 67: Cyclization of diketopiperazine 323 with a gold catalyst.

V. Experimental procedures

1. General methods

Commercially available solvents and reagents were used as such without further purification unless stated otherwise.

1.1. Solvents

Dry diethyl ether (Et_2O), tetrahydrofuran (THF) and toluene were freshly distilled from sodium or sodium/benzophenone ketyl. Dry dichloromethane (CH_2Cl_2) was freshly distilled over calcium hydride prior to use. Acetonitrile (ACN) was dried over 4Å molecular sieves. Methanol was dried by distillation over magnesium. Dry *N*,*N*-dimethylformamide (DMF) was obtained by distillation from magnesium sulfate and stored over 4Å molecular sieves.

1.2. Column chromatography

Purification by normal-phase column chromatography was performed in a glass column with silica gel (Aldrich, particle size 70-200 µm, pore diameter ca. 6 nm). Solvent systems were determined *via* thin layer chromatography (TLC) on glass plates coated with silica gel (Merck, Kieselgel $60F_{254}$, precoated 0.25 mm). Visualization was performed by UV irradiation (254 nm and 366 nm), oxidation by a $KMnO_4$ solution or elemental iodine. Reversed-phase column chromatography was performed on a Reveleris® X2 Flash Chromatography System with a Reveleris® C18 RP cartridge.

1.3. Preparative TLC

Preparative TLC was executed with TLC-plates (Analtech, Uniplate, 2000 µm 20 × 20 cm) in an elution chamber using an appropriate eluent.

1.4. Liquid chromatography

HPLC and HPLC-MS analysis were performed on an Agilent 1200 Series liquid chromatograph with a reversed-phase column (Eclipse plus C18 column, 50 × 4.6 mm, particle size 3.5 µm or a Supelco Ascentis Express C18 column, 30 × 4.6 mm, particle size 2.7 µm) connected to a UV/VIS detector and an Agilent 1100 Series LC/MSD type SL mass spectrometer with electrospray ionization (ESI, 70 eV) using a mass selective single quadrupole detector. A mixture of 5 mM NH_4OAc in H_2O and ACN was used as eluent.

1.5. Preparative HPLC

Preparative HPLC was performed on an Agilent 1100 Series liquid chromatograph using a reversed phase column (Zorbax Eclipse XDB-C18 column, 150 × 21.2 mm, particle size 5 µm) that is thermostatized at 25 °C. The column is connected to a UV-VIS Variable Wavelength Detector (VWD). A mixture of H_2O and ACN was used as eluent.

1.6. Mass spectrometry

Low-resolution mass spectra were recorded with an Agilent 1100 Series LC/MSD type SL mass spectrometer with electrospray ionization (ESI, 70 eV) using a mass selective single quadrupole detector. High-resolution mass spectra were obtained with an Agilent Technologies 6210 Time-Of-Flight (TOF) mass spectrometer, equipped with ESI/APCI-multimode source.

1.7. NMR spectroscopy

High resolution ^{1}H-NMR (300 or 400 MHz) and ^{13}C-NMR (75 or 100 MHz) spectra were recorded on a Jeol Eclipse FT 300 NMR spectrometer or a Bruker Avance III Nanobay 400 MHz spectrometer at room temperature, unless otherwise noted. Peak were assigned with the aid of DEPT, COSY, HSQC, HMBC, H2BC and NOE experiments. The compounds were diluted in deuterated solvents with tetramethylsilane (TMS) as an internal standard. All chemical shifts are expressed as parts per million (ppm).

1.8. Infrared spectroscopy

Infrared spectra were recorded on a Perkin Elmer Spectrum BX FT-IR spectrophotometer with an ATR (Attenuated Total Reflectance) accessory. All compounds were analyzed in neat form and only selected absorbances (ν_{max}, cm^{-1}) were reported.

1.9. Melting point

Melting points of crystalline compounds were determined using a Büchi B-540 apparatus or a Kofler bench, type WME Heizbank of Wagner & Munz (T_{max} 260 °C).

1.10. Microwave irradiation

All microwave reactions were performed in a CEM Discover Benchmate with a continuous power output from 0 to 300 Watt and a self-adjusting, single mode microwave cavity. The reactions were performed in 10 mL thick-walled Pyrex reaction vessels, closed with a snap-cap and equipped with a small magnetic stirring bar. A ramp time of maximum five minutes was used during which the temperature was increased from room temperature to the desired temperature. This temperature

was maintained during the course of the reaction for the indicated time. The temperature control system used an external infrared sensor to measure the temperature on the bottom of the vessel and was used in a feedback loop with the on-board computer to regulate the temperature from 25 to 250 °C by adjusting the power output (1 Watt increments). The pressure control, IntelliVent™ Pressure Control System, used an indirect measurement of the pressure by sensing changes in the external deflection of the septa on the top of the sealed pressure vessel. Stirring was performed by a rotating magnetic plate located below the floor of the microwave cavity. After the reaction the vial was cooled down by a stream of air onto the vial, which decreased the temperature of the vial from approximately 150 °C to 40 °C in less than 120 seconds.

1.11. Optical rotation

Optical rotations were recorded with a Jasco P-2000 polarimeter.

1.12. X-ray analysis

X-ray diffraction was performed using an Agilent Supernove Dual Source (Cu at zero) diffractometer equipped with an Atlas CCD detector using CuKα radiation (λ = 1.54178 Å) and ω scans. The images were interpreted and integrated with the program CrysAlisPro (Agilent Technologies). Using Olex2, the structure was solved by direct methods using the SheIXL program package. Non-hydrogen atoms were anisotropically refined and the hydrogen atoms in the riding mode and isotropic temperature factors fixed at 1.2 times U (eq) of the parent atoms. The amide and amine hydrogen atoms were located from a difference electron density map and were unrestrainedly refined.

All X-ray diffraction analyses were performed in collaboration with Prof. Dr. Kristof Van Hecke, XStruct, Department of Inorganic and Physical Chemistry, Ghent University, Belgium.

CCDC-1030976 and -1030977 contain the supplementary crystallographic data for this work and can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* https://summary.ccdc.cam.ac.uk/structure-summary-form.

2. Safety

2.1. General safety aspects

The practical work during this thesis was conducted in agreement with the SynBioC Research Group Internal Guidelines and the internal safety document "Safety Instructions: How to work with chemicals". All reactions were performed under a chemical fume hood, wearing protective clothes and eye protection.

2.2. Specific safety aspects

The Material Safety Data Sheet (MSDS) of the chemical provides a list of all the associated risks and procedures for handling that substance in a safe manner. These MSDS can be found on the website of the supplier. In certain cases reagents that show high risks were handled. The most important reagents are mentioned below.

Halogenated solvent (dichloromethane and chloroform): cause damage to organs through prolonged or repeated exposure. Toxic if inhaled and the vapors may cause drowsiness or dizziness. Breathing the vapors should be avoided. Release in the environment was avoided by separately collecting the solvents as well as all aqueous phases that had been in contact with halogenated solvents.

Non-halogenated solvent in general (THF, acetone, acetonitrile, methanol, …): are commonly used solvents. They cause acute toxicity after inhalation, specific target organ toxicity following single or repeated exposure. Keep them away from heat, fire, hot surfaces, sparks and ignition sources. Avoid inhalation and wear protective gloves and clothing.

Organic acids (acetic acid, formic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, …): are corrosive substances that can cause severe skin burns and eye damage.

Inorganic acids (HCl, …): are corrosive substances that can cause severe skin burns and eye damage and are corrosive to metals. The volatile acids may cause respiratory irritation. Wear protective gloves and protective clothing.

Hydrogen gas: extremely flammable gas. The gas was used under pressure (5 bar) and it may explode if heated. Keep it away from heat, fire, hot surfaces, sparks and ignition sources.

Sodium hydride (NaH): Do not allow contact with water. In contact with water releases flammable hydrogen gases which may ignite spontaneously. Keep away from sources of ignition.

Di- or triphosgene: Exposure to di- or triphosgene is similar in hazard to phosgene. The product is a corrosive material. Fatal if swallowed or inhaled and causes severe skin burns and eye damage (lachrymator). Decomposes in contact with water.

Palladium-based catalysts: highly flammable solids. They should be kept away from heat, sparks, open flames or hot surfaces.

EDC·HCl: causes serious eye damage and skin irritation. May cause respiratory irritation so avoid breathing dust of the compound. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals. It is considered a greener alternative to *N*,*N*'-dicyclohexylcarbodi-imide.[244]

Benzotriazole: is harmful if swallowed or if inhaled and causes serious eye irritation. The reagent is harmful to aquatic life with long lasting effects so release to the environment should be avoided.

Thionyl chloride: is a corrosive substance which causes severe skin burns and eye damage. It reacts violently with water and liberates a toxic gas.

3. Diketopiperazine synthesis *via* benzotriazole-assisted coupling

3.1. Synthesis of *N*-Cbz-D-Trp 155b[140]

D-Tryptophan (1.0 equiv., 49 mmol, 10.0 g) was suspended in H_2O (300 mL) and K_2CO_3 (2.0 equiv., 98 mmol, 13.5 g) and $NaHCO_3$ (1.0 equiv., 49 mmol, 4.11 g) were added. The addition of acetone (40 mL) gave a clear solution. CbzCl (1.25 equiv., 61 mmol, 8.7 mL) was slowly added to the solution while being cooled with an ice-water bath. Next, the mixture was warmed to 30 °C and after stirring for 3 hours the mixture was extracted with Et_2O (50 mL). The aqueous layer was acidified to a pH of 2 with 2 M HCl. The resulting precipitate was extracted by EtOAc. The organic phase was washed with H_2O (100 mL), dried over magnesium sulfate and the solids were removed by filtration. The solution was concentrated under reduced pressure and provided a viscous oil. The oil was redissolved in CH_2Cl_2 and evaporated. *N*-Cbz-D-Trp **155b** was obtained as a white powder (15.6 g, 94%) and was used in the next step without further purification.

3.2. Benzotriazole-activation of N-protected amino acids[130]

Thionyl chloride (1.1 equiv.) was added to a solution of 1*H*-benzotriazole (2.0 equiv.) in dry THF, and the reaction mixture was stirred for 20 min. The appropriate N-protected amino acid was added and the solution was stirred at room temperature for 2.5 hours. The solvent was then evaporated under reduced pressure and the residue was redissolved in CH_2Cl_2. The organic phase was washed twice with 2 M HCl and water and was dried over magnesium sulfate. Removal of the solvent under reduced pressure afforded the benzotriazole derivative as a yellow foam. The crude acylbenzotriazole was used for the coupling with an appropriate second amino acid.

3.3. Dipeptide synthesis with benzotriazole-activated amino acids[133]

A solution of benzotriazole-activated amino acid (1.0 equiv.), an appropriate second amino acid (1.0 equiv.) and triethylamine (1.0 equiv.) in dry ACN (4 mL/mmol amino acid) was subjected to microwave irradiation (70W, 50 °C, 10 min). Subsequently, the reaction mixture was concentrated under vacuum. The residue was redissolved in EtOAc and the solution was washed three times with 4 M HCl and brine. Then, the organic phase was dried over magnesium sulfate and the dipeptide was obtained as a yellow foam after removal of the solvent under reduced pressure. The crude dipeptide was again activated *via* the introduction of a benzotriazole group.

3.4. Benzotriazole activation of N-protected dipeptides[133]

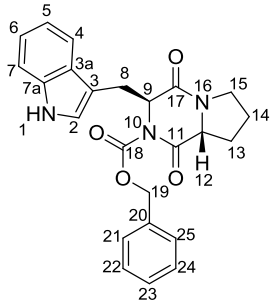
Thionyl chloride (1.0 equiv.) was added to a solution of 1*H*-benzotriazole (4.0 equiv.) in CH_2Cl_2, and the reaction mixture was stirred for 30 min. Next, the reaction mixture was cooled to -10 °C. The

appropriate dipeptide was added and stirred at -10 °C for one hour. Subsequently, the mixture was washed twice with 4 M HCl, twice with saturated aqueous $NaHCO_3$ and with brine before the organic phase was dried over magnesium sulfate. The organic phase was concentrated *in vacuo,* which afforded the desired benzotriazole-activated dipeptides as yellow-orange foams.

3.5. Trans-selective cyclization/epimerization for the synthesis of *trans*-cyclo(*N*-Cbz-Trp, Pro)

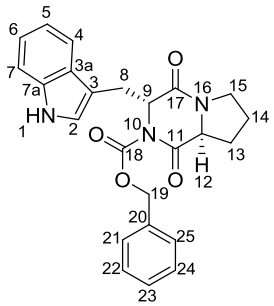
N-Cbz-protected dipeptidoyl benzotriazoles **163** were synthesized according to the general procedures (*see* 3.1 to 3.4). A solution of N-Cbz-protected dipeptidoyl benzotriazole **163** (1.0 equiv.) and triethylamine (1.0 equiv.) in dry ACN (4 mL/mmol **163**) was subjected to microwave irradiation (70W, 80 °C, 35 min). Subsequently, the reaction mixture was concentrated under vacuum.[137]

benzyl (3*S*,8a*R*)-3-((1*H*-indol-3-yl)methyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1*H*)-carboxylate 170a



The general procedure was applied on the dipeptide isomer **163a** (1.9 mmol, 1.0 g). The crude mixture was redissolved in CH_2Cl_2 (25 mL) and washed three times with 4 M HCl (3×15ml). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure, providing the *trans*-diketopiperazine **170a**. Spectral data are in accordance with reported values.[137, 245] **Yield** 96% (0.75 g); yellow foam; $[\alpha]_D^{21}$ = +135.8 (c=0.2 in CH_2Cl_2); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.11-1.28 (1H, m, C$\underline{H}_a$H$_b$-14), 1.60-1.79 (2H, m, C$\underline{H}_a$H$_b$-13, CH$_a\underline{H}_b$-14), 1.87-1.99 (1H, m, CH$_a\underline{H}_b$-13), 2.23-2.32 (1H, m, C$\underline{H}$-12), 3.04-3.14 (1H, m, C$\underline{H}_a$H$_b$-15), 3.36 (1H, dd, *J*=15.0 Hz, *J*=5.2 Hz, C$\underline{H}_a$H$_b$-8), 3.39-3.48 (1H, m, CH$_a\underline{H}_b$-15), 3.59 (1H, dd, *J*=15.0 Hz, *J*=3.4 Hz, CH$_a\underline{H}_b$-8), 5.10 (1H, dd, *J*=5.2 Hz, *J*=3.4 Hz, C$\underline{H}$-9), 5.22 (1H, d, *J*=12.1 Hz, C$\underline{H}_a$H$_b$-19), 5.34 (1H, d, *J*=12.1 Hz, CH$_a\underline{H}_b$-19), 6.89 (1H, s, C$\underline{H}$-2), 7.09 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.18 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-6), 7.31-7.43 (6H, m, C$\underline{H}$-7, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24, C$\underline{H}$-25), 7.53 (1H, d, *J*=7.5 Hz, C$\underline{H}$-4), 8.28 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.6 (CH_2-14), 28.4 (CH_2-8), 29.3 (CH_2-13), 44.9 (CH_2-15), 58.9 (CH-12), 62.1 (CH-9), 69.1 (CH_2-19), 109.1 (C_q-3), 111.3 (CH-7), 118.9 (CH-4), 119.9 (CH-5), 122.6 (CH-6), 124.6 (CH-2), 127.1 (C_q-3a), 128.4 (CH-21, CH-25), 128.7 (CH-22, CH-23, CH-24), 134.7 (C_q-20), 136.1 (C_q-7a), 152.1 ($C_{C=O}$-18), 164.9 ($C_{C=O}$-17), 167.9 ($C_{C=O}$-11) ppm.

benzyl (3*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1*H*)-carboxylate 170b



The general procedure was applied on the dipeptide isomer **163b** (3.7 mmol, 2.0 g). The crude mixture was directly purified by column chromatography (3/7 petroleum ether/EtOAc) to give the corresponding *trans*-diketopiperazine **170b**.[245] **Yield** 41% (0.64 g); yellow foam; R_f=0.21 (3/7 petroleum ether/EtOAc); $[\alpha]_D^{21}$ = -135.0 (c=0.2 in CH_2Cl_2); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.09-1.28 (1H, m, C$\underline{H}_a$H$_b$-14), 1.59-1.79 (2H, m, C$\underline{H}_a$H$_b$-13, CH$_a\underline{H}_b$-14), 1.86-1.95 (1H, m, CH$_a\underline{H}_b$-13), 2.33 (1H, dd, *J*=10.2 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 3.05-3.15 (1H, m, C$\underline{H}_a$H$_b$-15), 3.36 (1H, dd, *J*=15.0 Hz, *J*=5.3 Hz, C$\underline{H}_a$H$_b$-8), 3.39-3.48 (1H, m, CH$_a\underline{H}_b$-15), 3.57 (1H, dd, *J*=15.0 Hz, *J*=3.8 Hz, CH$_a\underline{H}_b$-8), 5.11 (1H, dd, *J*=5.3 Hz, *J*=3.8 Hz, C$\underline{H}$-9), 5.19 (1H, d, *J*=12.1 Hz, C$\underline{H}_a$H$_b$-19), 5.31 (1H, d, *J*=12.1 Hz, CH$_a\underline{H}_b$-19), 6.86 (1H, s, C$\underline{H}$-2), 7.07 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.16 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-6), 7.27-7.43 (6H, m, C$\underline{H}$-7, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24, C$\underline{H}$-25), 7.52 (1H, d, *J*=7.5 Hz, C$\underline{H}$-4), 8.76 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.7 (CH_2-14), 28.6 (CH_2-8), 29.4 (CH_2-13), 45.0 (CH_2-15), 59.0 (CH-12), 62.2 (CH-9), 69.2 (CH_2-19), 108.9 (C_q-3), 111.5 (CH-7), 118.8 (CH-4), 119.9 (CH-5), 122.5 (CH-6), 124.8 (CH-2), 127.2 (C_q-3a), 128.5 (CH-21, CH-25), 128.8 (CH-22, CH-23, CH-24), 134.7 (C_q-20), 136.2 (C_q-7a), 152.0 ($C_{C=O}$-18), 165.0 ($C_{C=O}$-17), 168.1 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 3345 (NH), 1774 (C=O), 1725 (C=O), 1657 (C=O); **MS** (ES): *m/z* (%): 374 (100) [M - CO_2 + H]$^+$; **HRMS** (ESI): calcd. for $C_{24}H_{24}N_3O_4^+$ [M+H]$^+$: 418.1761; found: 418.1874.

4. Diketopiperazine synthesis via carbodiimide-assisted coupling

4.1. Synthesis of the cyclo(Trp, Pro) isomers

4.1.1. EDC-assisted coupling

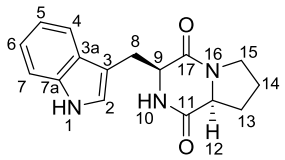
Proline methyl ester hydrochloride **183** (1 equiv.) was dissolved in anhydrous CH_2Cl_2 and *N*-benzyloxycarbonyltryptophan **155** (1 equiv.), EDC·HCl (1 equiv.) and triethylamine (1 equiv.) were subsequently added under a nitrogen atmosphere. The reaction was stirred at room temperature for 24 hours and was then washed three times with 1 M HCl and saturated aq. $NaHCO_3$. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure, yielding dipeptide **181** as a yellow foam.[125]

4.1.2. Hydrogenolysis and cyclization

To a solution of dipeptide **181** in MeOH, 5 wt% of Pd/C was added. The reaction mixture was stirred under 5 atm of H_2 for 2-3 hours at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. In the case of the D,L- and L,D-isomers, the methanolic solution was stirred at room temperature until ring closure was complete. In the case of the *cis*-fused isomers, ammonia in methanol (7 M NH_3) was added to induce ring formation. The reaction were monitored *via* HPLC-MS. The filtrate was concentrated *in vacuo* to give the crude diketopiperazine **2**. The pure product **2** was obtained after recrystallization from methanol as colourless crystals. The structure of the products was confirmed by comparison of the spectroscopic data with literature values.[58, 115, 121]

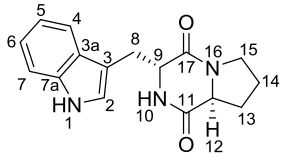
It should be noted that for further reactions with **2** it is advisable to redissolve the DKP **2** crystals in methanol and evaporate the solvent under reduced pressure to obtain **2** in its amorphic 'foam' state. That way the compound **2** dissolves more readily in the solvent used in the subsequent reaction.

(3*S*,8a*S*)-3-((1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione 2a



Following general procedures 4.1.1 and 4.1.2 on a 20 mmol scale, isomer **2a** was obtained from **155a** and **183a** in 3 steps. **Yield** 70% (3.97 g); colourless crystals; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.85-2.10 (3H, m, C$\underline{H}_a$H$_b$-13, C$\underline{H}_2$-14); 2.28-2.39 (1H, m, CH$_a\underline{H}_b$-13); 2.97 (1H, dd, *J*=15.1 Hz, 10.7 Hz, C$\underline{H}_a$H$_b$-8); 3.57-3.68 (2H, m, C$\underline{H}_2$-15); 3.72-3.81 (1H, m, CH$_a\underline{H}_b$-8); 4.08 (1H, ddd, *J*=8.0 Hz, 8.0 Hz, 1.10 Hz, C$\underline{H}$-12); 4.38 (1H, dd, *J*=10.7 Hz, 2.5 Hz, C$\underline{H}$-9); 5.73 (1H, br s, N$\underline{H}$-10); 7.11 (1H, d, *J*=2.2 Hz, C$\underline{H}$-2); 7.15 (1H, ddd, *J*=7.7 Hz, 7.7 Hz, 1.10 Hz, C$\underline{H}$-5); 7.24 (1H, ddd, *J*=7.7 Hz, 7.7 Hz, 1.7 Hz, C$\underline{H}$-6); 7.40 (1H, br d, *J*=7.7 Hz, C$\underline{H}$-7); 7.59 (1H, br d, *J*=7.7 Hz, C$\underline{H}$-4); 8.26 (1H, br s, N$\underline{H}$-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 22.7 (CH_2-14); 26.9 (CH_2-8); 28.4 (CH_2-13); 45.5 (CH_2-15); 54.6 (CH-9); 59.3 (CH-12); 110.1 (C_q-3); 111.7 (CH-7); 118.6 (CH-4); 120.1 (CH-5); 122.9 (CH-6); 123.4 (CH_2); 126.8 (C_q-3a); 136.8 (C_q-7a); 165.6 ($C_{C=O}$-17); 169.4 ($C_{C=O}$-11) ppm.

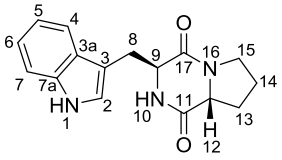
(3*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione 2b



Following general procedure 4.1.1 and 4.1.2 on a 46 mmol scale, isomer **2b** was obtained from **155b** and **183a** in 2 steps. **Yield** 79% (10.32 g); colourless crystals; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.33-1.47 (1H, m, C$\underline{H}_a$H$_b$-14), 1.64-1.76 (1H, m, C$\underline{H}_a$H$_b$-13), 1.78-1.87 (1H, m, CH$_a\underline{H}_b$-14), 2.01-2.10 (1H, m, CH$_a\underline{H}_b$-13), 2.78 (1H, dd, *J*=10.9 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 3.13-3.21 (1H, m, C$\underline{H}_a$H$_b$-15), 3.18 (1H, dd, *J*=14.5 Hz, *J*=4.3 Hz, C$\underline{H}_a$H$_b$-8), 3.41 (1H, dd, *J*=14.5 Hz, *J*=5.9 Hz, CH$_a\underline{H}_b$-8), 3.50-3.58 (1H, m, CH$_a\underline{H}_b$-15), 4.21-4.26 (1H, m, C$\underline{H}$-9), 6.36 (1H, br s, N$\underline{H}$-10), 7.03 (1H, s, C$\underline{H}$-2), 7.12 (1H, dd, *J*=7.5 Hz,

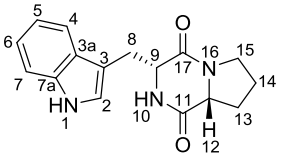
J=7.5 Hz, C<u>H</u>-5), 7.19 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C<u>H</u>-6), 7.36 (1H, d, *J*=7.5 Hz, C<u>H</u>-7), 7.61 (1H, d, *J*=7.5 Hz, C<u>H</u>-4), 8.46 (1H, br s, N<u>H</u>-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.4 (CH_2-14), 28.8 (CH_2-13), 30.4 (CH_2-8), 45.0 (CH_2-15), 57.8 (CH-12), 58.2 (CH-9), 108.9 (C_q-3), 111.4 (CH-7), 118.8 (CH-4), 119.6 (CH-5), 122.4 (CH-6), 124.6 (CH-2), 127.1 (C_q-3a), 136.2 (C_q-7a), 165.9 ($C_{C=O}$-17), 170.0 ($C_{C=O}$-11) ppm.

(3*S*,8a*R*)-3-((1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione 2c



Following general procedure 4.1.1 and 4.1.2 on a 10 mmol scale, isomer **2c** was obtained from **155a** and **183b** in 2 steps. **Yield** 74% (2.10 g); colourless crystals; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.38-1.49 (1H, m, C<u>H$_a$</u>H$_b$-14), 1.67-1.76 (1H, m, C<u>H$_a$</u>H$_b$-13), 1.80-1.89 (1H, m, CH$_a$<u>H$_b$</u>-14), 2.05-2.13 (1H, m, CH$_a$<u>H$_b$</u>-13), 2.86 (1H, dd, *J*=10.7 Hz, *J*=6.6 Hz, C<u>H</u>-12), 3.15-3.23 (1H, m, C<u>H$_a$</u>H$_b$-15), 3.20 (1H, d, *J*=14.6 Hz, C<u>H$_a$</u>H$_b$-8), 3.41 (1H, dd, *J*=14.6 Hz, *J*=6.1 Hz, CH$_a$<u>H$_b$</u>-8), 3.52-3.61 (1H, m, CH$_a$<u>H$_b$</u>-15), 4.22-4.27 (1H, m, C<u>H</u>-9), 5.86 (1H, s, N<u>H</u>-10), 7.06 (1H, s, C<u>H</u>-2), 7.14 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C<u>H</u>-5), 7.21 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C<u>H</u>-6), 7.36 (1H, d, *J*=7.5 Hz, C<u>H</u>-7), 7.62 (1H, d, *J*=7.5 Hz, C<u>H</u>-4), 8.17 (1H, s, N<u>H</u>-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.6 (CH_2-14), 29.0 (CH_2-13), 30.6 (CH_2-8), 45.1 (CH_2-15), 58.0 (CH-12), 58.4 (CH-9), 109.2 (C_q-3), 111.4 (CH-7), 118.9 (CH-4), 119.8 (CH-5), 122.4 (CH-6), 124.5 (CH-2), 127.2 (C_q-3a), 136.3 (C_q-7a), 165.8 ($C_{C=O}$-17), 170.0 ($C_{C=O}$-11) ppm.

(3*R*,8a*R*)-3-((1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione 2d



Following general procedure 4.1.1 and 4.1.2 on a 10 mmol scale, isomer **2d** was obtained from **155b** and **183b** in 3 steps. **Yield** 63% (1.78 g); colourless crystals; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.86-2.07 (3H, m, C<u>H$_a$</u>H$_b$-13, C<u>H$_2$</u>-14), 2.27-2.38 (1H, m, CH$_a$<u>H$_b$</u>-13), 2.97 (1H, dd, *J*=14.9 Hz, *J*=10.9 Hz, C<u>H$_a$</u>H$_b$-8), 3.53-3.69 (2H, m, C<u>H$_2$</u>-15), 3.57 (1H, dd, *J*=14.9 Hz, *J*=3.4 Hz, CH$_a$<u>H$_b$</u>-8), 4.07 (1H, t, *J*=7.7 Hz, C<u>H</u>-12), 4.37 (1H, dd, *J*=10.9 Hz, *J*=3.4 Hz, C<u>H</u>-9), 5.79 (1H, s, N<u>H</u>-10), 7.07 (1H, d, *J*=2.2 Hz, C<u>H</u>-2), 7.14 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C<u>H</u>-5), 7.23 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C<u>H</u>-6), 7.39 (1H, d, *J*=7.8 Hz, C<u>H</u>-7), 7.59 (1H, d, *J*=7.8 Hz, C<u>H</u>-4), 8.45 (1H, s, N<u>H</u>-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 22.7 (CH_2-14), 26.9 (CH_2-8), 28.4 (CH_2-13), 45.5 (CH_2-15), 54.7 (CH-9), 59.3 (CH-12), 109.9 (C_q-3), 111.7 (CH-7), 118.6 (CH-4), 120.0 (CH-5), 122.8 (CH-6), 123.5 (CH-2), 126.8 (C_q-3a), 136.8 (C_q-7a), 165.7 ($C_{C=O}$-17), 169.5 ($C_{C=O}$-11) ppm.

4.2. Synthesis of the cyclo(Trp, Hyp) isomers

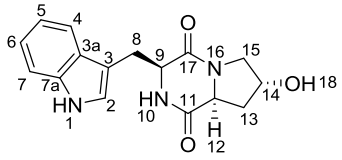
4.2.1. EDC-assisted coupling

Trans-4-hydroxy-L-proline methyl ester hydrochloride **299** (1 equiv., 10 mmol, 1.82 g) was dissolved in dry CH_2Cl_2 (80 mL) and *N*-benzyloxycarbonyltryptophan **155** (1 equiv., 10 mmol, 3.38 g), EDC·HCl (1 equiv., 10 mmol, 1.96 g) and triethylamine (1 equiv., 10 mmol, 1.4 mL) were subsequently added under a nitrogen atmosphere. The reaction was stirred at room temperature for 24 hours and was then washed three times with 1 M HCl (80 mL) and saturated aq. $NaHCO_3$ (80 mL). The organic layer was dried over magnesium sulfate and concentrated, yielding dipeptide **182** as a white foam.[125]

4.2.2. Hydrogenolysis and cyclization

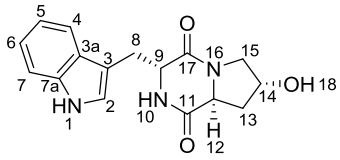
To a solution of dipeptide **182** in MeOH (200 mL), 5 wt% of Pd/C was added. The reaction mixture was stirred under 5 atm of H_2 for 2 hours at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. In the case of the D,L-isomer **182b**, the methanolic solution was stirred at room temperature until ring closure was complete. In the case of the L,L-isomer **182a**, ammonia in methanol (7 M NH_3) was added to induce ring formation. The reaction was monitored *via* HPLC-MS. The filtrate was concentrated *in vacuo* to give the crude diketopiperazine **190**, which was purified *via* column chromatography or recrystallization.

(3*S*,7*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione 190a



Applying general procedures 4.2.1 and 4.2.2 on **299** and **155a**, isomer **190a** was obtained after column chromatography. **Yield** 40% (1.20 g); white amorphous powder; R_f=0.14 (3/1 EtOAc/acetone); m.p. 146-150 °C; $[\alpha]_D^{25}$ = -74.2 (c=0.44 in DMSO); **^{1}H-NMR (400 MHz, DMSO-d6) δ** 1.47 (1H, dd, *J*=12.7 Hz, *J*=11.3 Hz, *J*=4.6 Hz, C$\underline{H}_a$H$_b$-13), 1.90 (1H, dd, *J*=12.7 Hz, *J*=6.3 Hz, CH$_a$$\underline{H}_b$-13), 3.07 (1H, dd, *J*=14.9 Hz, *J*=5.8 Hz, C$\underline{H}_a$H$_b$-8), 3.15 (1H, d, *J*=12.4 Hz, C$\underline{H}_a$H$_b$-15), 3.25 (1H, dd, *J*=14.9 Hz, *J*=4.7 Hz, CH$_a$$\underline{H}_b$-8), 3.51 (1H, dd, *J*=12.4 Hz, *J*=4.8 Hz, CH$_a$$\underline{H}_b$-15), 4.08-4.13 (1H, m, C$\underline{H}$-14), 4.30 (1H, dd, *J*=11.3 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 4.34-4.38 (1H, m, C$\underline{H}$-9), 5.11 (1H, d, *J*=3.3 Hz, O$\underline{H}$-18), 6.97 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-5), 7.06 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-6), 7.18 (1H, d, *J*=2.3 Hz, C$\underline{H}$-2), 7.33 (1H, d, *J*=7.6 Hz, C$\underline{H}$-7), 7.57 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4), 7.72 (1H, s, N$\underline{H}$-10), 10.85 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (100 MHz, DMSO-d6) δ** 26.2 (CH_2-8), 37.6 (CH_2-13), 54.3 (CH_2-15), 55.6 (CH-9), 57.4 (CH-12), 67.2 (CH-14), 109.7 (C_q-3), 111.7 (CH-7), 118.7 (CH-5), 119.1 (CH-4), 121.4 (CH-6), 124.8 (CH-2), 127.8 (C_q-3a), 136.4 (C_q-7a), 166.1 ($C_{C=O}$-17), 169.9 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 3263 (NH), 1644 (C=O), 1420; **MS** (ES): *m/z* (%): 300 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{18}N_3O_3^+$ $[M+H]^+$: 300.1343; found: 300.1336.

(3*R*,7*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione 190b

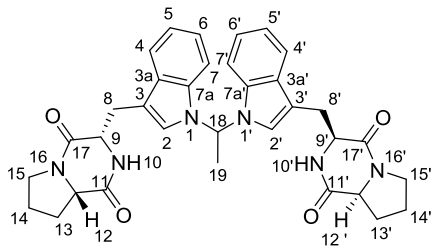


Applying general procedures 4.2.1 and 4.2.2 on **299** and **155b**, isomer **190b** was obtained after recrystallization from methanol. **Yield** 40% (1.20 g); colourless crystals; m.p. >260 °C; $[\alpha]_D^{25}$ = -56.6 (c=0.44 in DMSO); **^{1}H-NMR (400 MHz, DMSO-d6) δ** 1.68 (1H, dd, *J*=12.5 Hz, *J*=11.8 Hz, *J*=4.4 Hz, C$\underline{H}_a$H$_b$-13), 1.83 (1H, dd, *J*=12.5 Hz, *J*=6.2 Hz, CH$_a\underline{H}_b$-13), 2.93 (1H, d, *J*=12.6 Hz, C$\underline{H}_a$H$_b$-15), 3.04 (1H, dd, *J*=14.4 Hz, *J*=4.8 Hz, C$\underline{H}_a$H$_b$-8), 3.18 (1H, dd, *J*=14.4 Hz, *J*=6.0 Hz, CH$_a\underline{H}_b$-8), 3.38 (1H, dd, *J*=11.8 Hz, *J*=6.2 Hz, C$\underline{H}$-12), 3.58 (1H, dd, *J*=12.6 Hz, *J*=4.9 Hz, CH$_a\underline{H}_b$-15), 3.93-3.98 (1H, m, C$\underline{H}$-9), 4.12-4.17 (1H, m, C$\underline{H}$-14), 4.84 (1H, d, *J*=2.8 Hz, O$\underline{H}$-18), 6.96 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, C$\underline{H}$-5), 7.05 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, C$\underline{H}$-6), 7.09 (1H, d, *J*=2.3 Hz, C$\underline{H}$-2), 7.33 (1H, d, *J*=8.0 Hz, C$\underline{H}$-7), 7.84 (1H, d, *J*=8.0 Hz, C$\underline{H}$-4), 8.17 (1H, d, *J*=3.8 Hz, N$\underline{H}$-10), 10.92 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (100 MHz, DMSO-d6) δ** 29.0 (CH_2-8), 37.4 (CH_2-13), 53.4 (CH_2-15), 55.0 (CH-12), 57.3 (CH-9), 65.4 (CH-14), 107.9 (C_q-3), 110.7 (CH-7), 117.7 (CH-4), 117.8 (CH-5), 120.3 (CH-6), 123.7 (CH-2), 126.8 (C_q-3a), 135.4 (C_q-7a), 164.8 ($C_{C=O}$-17), 168.0 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 3216 (NH), 1693 (C=O), 1628 (C=O), 1455; **MS** (ES): *m/z* (%): 300 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{18}N_3O_3^+$ $[M+H]^+$: 300.1343; found: 300.1341.

5. Synthesis of dimers *via* the Pictet-Spengler reaction

General procedure: Diketopiperazine **2** (2 equiv.) was dissolved in dry CH_2Cl_2 at room temperature under a nitrogen atmosphere. The aldehyde (2.5 equiv.) was added and the mixture was cooled to -40 °C before trifluoroacetic acid (20 equiv.) was added. The mixture was stirred for one hour at -40 °C. The reaction mixture was quenched by the careful addition of saturated $NaHCO_3$ solution and subsequently washed twice with saturated $NaHCO_3$ solution and brine. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. Purification of the foam residue by chromatography provided the desired products.

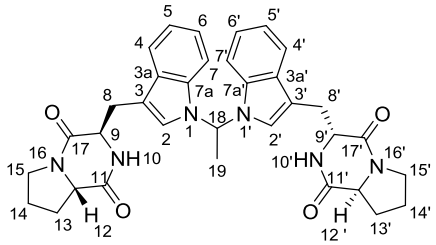
(3*S*,3'*S*,8a*S*,8a'*S*)-3,3'-((ethane-1,1-diylbis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 199a



Using the general procedure with diketopiperazine isomer **2a** and acetaldehyde on a 0.7 mmol scale, compound **199a** was obtained after column chromatography. **Yield** 46% (0.191 g); yellow powder; R_f=0.01 (CH_2Cl_2/2% MeOH); m.p. 164-168 °C; $[\alpha]_D^{25}$ = -135.8 (c=0.72 in CH_2Cl_2); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 0.11-0.26 (1H, m, C$\underline{H}_a$H$_b$-13); 1.11-1.22 (1H, m, C$\underline{H}_a$H$_b$-14);

1.34-1.48 (2H, m, CH_aH_b-13, CH_aH_b-14); 1.61-1.69 (1H, m, CH_aH_b-13‘); 1.79-1.89 (2H, m, CH_2-14‘); 2.07 (3H, d, *J*=6.6 Hz, CH_3-19); 2.26-2.37 (1H, m, CH_aH_b-13‘); 3.09-3.18 (1H, m, CH_aH_b-15); 3.19 (1H, dd, *J*=14.9 Hz, *J*=4.7 Hz, CH_aH_b-8‘); 3.33-3.45 (3H, m, CH_2-8, CH_aH_b-15); 3.45-3.69 (4H, m, CH_aH_b-8‘,CH-12, CH_2-15‘); 4.05-4.17 (1H, m, CH-12‘); 4.34 (1H, br dd, *J*=4.7 Hz, *J*=4.7 Hz, CH-9‘); 4.47 (1H, br dd, *J*=4.4 Hz, *J*=4.4 Hz, CH-9); 6.85 (1H, br s, CH-2); 6.92 (1H, q, *J*=6.6 Hz, CH-18); 7.02 (1H, br s, CH-2‘); 7.08-7.27 (4H, m, CH-5, CH-5‘,CH-6, CH-6‘); 7.36 (1H, d, *J*=9.1 Hz, CH-7‘); 7.39 (1H, d, *J*=9.1 Hz, CH-7); 7.59-7.68 (3H, m, NH-10‘,CH-4, CH-4‘); 8.09 (1H, br s, NH-10) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.2 (CH_3-19); 21.4 (CH_2-14); 22.3 (CH_2-14‘); 26.9 (CH_2-8); 28.1 (CH_2-13); 28.8 (CH_2-13‘); 29.1 (CH_2-8‘); 44.9 (CH_2-15); 45.4 (CH_2-15‘); 55.1 (CH-9); 55.5 (CH-9‘); 58.6 (CH-12); 59.2 (CH-12‘); 61.4 (CH-18); 108.7 (C_q-3); 109.5 (C_q-3‘); 110.3 (CH-7); 110.4 (CH-7‘); 119.6 (CH-4); 120.2 (CH-5); 120.4 (CH-5‘); 120.5 (CH-4‘); 122.4 (CH-2); 122.6 (CH-6); 123.0 (CH-6‘); 123.6 (CH-2‘); 127.6 (C_q-3a); 129.2 (C_q-3a‘); 135.7 (C_q-7a); 135.8 (C_q-7a‘); 164.7 ($C_{C=O}$-11*); 165.5 ($C_{C=O}$-11‘*); 169.0 ($C_{C=O}$-17*); 170.2 ($C_{C=O}$-17‘*) ppm (The allocation of the signals CH_y-X and CH_y-X‘ as well as signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 1655 (C=O), 1455; **MS** (ES): *m/z* (%): 593 (100) $[M + H]^+$, 310 (70) $[M–DKP+H]^+$; **HRMS** (ESI): calcd. for $C_{34}H_{37}N_6O_4^+$ $[M+H]^+$: 593.2871; found: 593.2863.

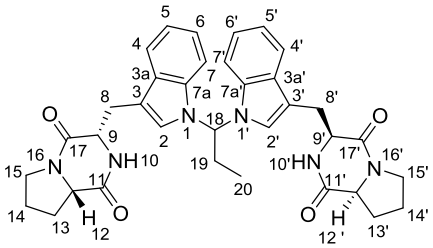
(3*R*,3'*R*,8a*S*,8a'*S*)-3,3'-((ethane-1,1-diylbis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 199b



Using the general procedure with diketopiperazine isomer **2b** and acetaldehyde on a 1.0 mmol scale, compound **199b** was obtained after reversed-phase chromatography using a H_2O/ACN gradient (2 column volumes (CVs) 15% ACN, over 30 CVs to 40% ACN). **Yield** 32% (0.19 g); white foam; m.p. 184-188 °C; $[\alpha]_D^{25}$ = +122.7 (c=0.72 in CH_2Cl_2); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 84-1.12 (2H, m, CH_aH_b-13, CH_aH_b-14), 1.14-1.22 (1H, m, CH_aH_b-13), 1.24-1.31 (1H, m, CH-12), 1.50-1.59 (1H, m, CH_aH_b-14), 1.79-2.09 (3H, m, CH_aH_b-13’, CH_2-14’), 2.10 (3H, d, *J*=6.6 Hz, CH_3-19), 2.34-2.43 (1H, m, CH_aH_b-13’), 2.76 (1H, ddd, *J*=12.0 Hz, *J*=9.6 Hz, *J*=2.5 Hz, CH_aH_b-15), 2.93 (1H, dd, *J*=14.8 Hz, *J*=5.0 Hz, CH_aH_b-8), 3.05 (1H, dd, *J*=14.2 Hz, *J*=11.1 Hz, CH_aH_b-8’), 3.30 (1H, dt, *J*=12.0 Hz, *J*=8.6 Hz, CH_aH_b-15), 3.47-3.58 (3H, m, CH_aH_b-8, CH_aH_b-8’, CH_aH_b-15’), 3.70 (1H, dt, *J*=11.8 Hz, *J*=8.5 Hz, CH_aH_b-15’), 3.88 (1H, dd, *J*=10.1 Hz, *J*=6.6 Hz, CH-12’), 4.16-4.20 (1H, m, CH-9), 4.52 (1H, ddd, *J*=11.1 Hz, *J*=4.2 Hz, *J*=4.2 Hz, CH-9’), 6.77 (1H, s, CH-2), 6.96 (1H, q, *J*=6.6 Hz, CH-18), 7.09-7.18 (3H, m, CH-5, CH-5’, CH-6’), 7.20 (1H, s, CH-2’), 7.22 (1H, d, *J*=7.7 Hz, CH-7’), 7.32 (1H, ddd, *J*=7.9 Hz, *J*=7.9 Hz, *J*=0.9 Hz, CH-6), 7.55 (1H, d, *J*=7.9 Hz, CH-4), 7.59 (1H, d, *J*=7.9 Hz, CH-7), 7.71 (1H, d, *J*=7.7 Hz, CH-4’), 7.97 (1H, d, *J*=2.9 Hz, NH-10), 9.05 (1H, d, *J*=4.2 Hz, NH-10’) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 21.1 (CH_2-14), 21.2 (CH_3-19), 22.1 (CH_2-14’), 28.2 (CH_2-13), 29.1

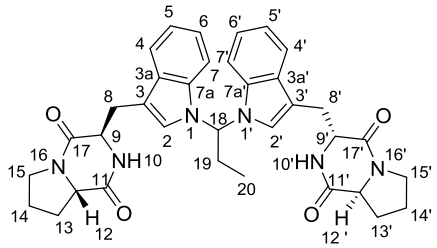
(CH_2-13'), 30.2 (CH_2-8), 30.6 (CH_2-8'), 44.6 (CH_2-15), 45.4 (CH_2-15'), 56.3 (CH-9'), 57.0 (CH-12), 57.3 (CH-9), 58.3 (CH-12'), 60.5 (CH-18), 107.9 (CH-7), 108.7 (CH-7'), 110.1 (C_q-3), 110.9 (C_q-3'), 119.8 (CH-4), 120.0 (CH-5), 120.1 (CH-4'), 120.7 (CH-5'), 122.6 (CH-6'), 123.0 (CH-6), 123.1 (CH-2'), 123.4 (CH-2), 127.4 (C_q-3a), 128.0 (C_q-3a'), 135.3 (C_q-7a), 136.4 (C_q-7a'), 165.1 ($C_{C=O}$-17), 166.1 ($C_{C=O}$-17'), 170.2 ($C_{C=O}$-11), 170.6 ($C_{C=O}$-11') ppm (The allocation of the signals CH_y-X and CH_y-X' may be interchanged); **IR** (cm^{-1}): υmax = 3224 (NH), 1651 (C=O), 1452; **MS** (ES): *m/z* (%): 593 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{34}H_{37}N_6O_4^+$ $[M+H]^+$: 593.2871; found: 593.2872.

(3*S*,3'*S*,8a*S*,8a'*S*)-3,3'-((propane-1,1-diylbis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 200a



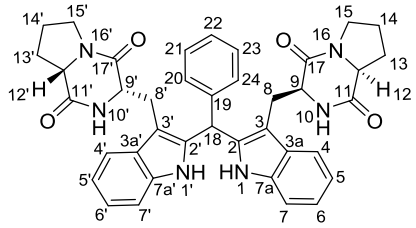
Using the general procedure with diketopiperazine isomer **2a** and propionaldehyde on a 0.7 mmol scale, compound **200a** was obtained after column chromatography. **Yield** 16% (0.068 g); yellow powder; R_f=0.01 (CH_2Cl_2/5% MeOH); m.p. 150-158 °C; $[\alpha]_D^{25}$ = -143.0 (c=0.42 in CH_2Cl_2); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** -0.29-(-0.15) (1H, m, C$\underline{H}_a$$H_b$-13); 0.87-0.97 (1H, m, C$\underline{H}_a$$H_b$-14); 0.97 (3H, t, *J*=7.2 Hz, C$\underline{H}_3$-20); 1.11 (1H, m, $CH_a\underline{H}_b$-13); 1.18-1.34 (1H, m, $CH_a\underline{H}_b$-14); 1.70-2.08 (3H, m, C$\underline{H}_a$$H_b$-13', C$\underline{H}_2$-14'); 2.29-2.59 (3H, m, $CH_a\underline{H}_b$-13', C$\underline{H}_2$-19); 3.02 (1H, ddd, *J*=11.6 Hz, *J*=10.0 Hz, *J*=4.0 Hz, C$\underline{H}_a$$H_b$-15); 3.11 (1H, dd, *J*=14.6 Hz, 4.7 Hz, C$\underline{H}_a$$H_b$-8'); 3.31 (1H, ddd, *J*=11.6 Hz, *J*=8.0 Hz, *J*=8.0 Hz, $CH_a\underline{H}_b$-15); 3.43 (2H, d, *J*=5.0 Hz, C$\underline{H}_2$-8); 3.49-3.64 (4H, m, $CH_a\underline{H}_b$-8', C$\underline{H}$-12, C$\underline{H}_2$-15'); 4.08-4.16 (1H, m, C$\underline{H}$-12'); 4.31 (1H, br s, C$\underline{H}$-9'); 4.49 (1H, dd, *J*=5.0 Hz, *J*=3.9 Hz, C$\underline{H}$-9); 6.60 (1H, dd, *J*=7.4 Hz, *J*=7.4 Hz, C$\underline{H}$-18); 6.95 (1H, s, C$\underline{H}$-2); 6.98 (1H, s, C$\underline{H}$-2'); 7.09 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5); 7.10 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5'); 7.16 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6); 7.24 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6'); 7.37 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7'); 7.47 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7); 7.60 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4); 7.65 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4'); 7.79 (1H, s, N$\underline{H}$-10); 8.36 (1H, s, N$\underline{H}$-10') ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 10.6 (CH_3-20); 21.1 (CH_2-14); 22.5 (CH_2-14'); 26.6 (CH_2-8); 27.9 (CH_2-13); 28.6 (CH_2-19*); 28.8 (CH_2-13'*); 29.6 (CH_2-8'); 44.8 (CH_2-15); 45.4 (CH_2-15'); 55.0 (CH-9); 55.7 (CH-9'); 58.4 (CH-12); 59.3 (CH-12'); 66.4 (CH-18); 108.6 (CH-7); 109.4 (CH-7'); 110.2 (C_q-3); 110.7 (C_q-3'); 119.5 (CH-4); 120.0 (CH-5); 120.4 (CH-5'); 120.6 (CH-4'); 122.2 (CH-2); 122.6 (CH-6); 122.9 (CH-6'); 123.5 (CH-2'); 127.4 (C_q-3a); 129.2 (C_q-3a'); 136.2 (C_q-7a); 136.4 (C_q-7a'); 164.6 ($C_{C=O}$-11**); 165.6 ($C_{C=O}$-11'**); 168.9 ($C_{C=O}$-17**); 170.4 ($C_{C=O}$-17'**) ppm (The allocation of the signals CH_y-X and CH_y-X' as well as signals with the same superscript (*, **) may be interchanged); **IR** (cm^{-1}): υmax = 1654 (C=O), 1454; **MS** (ES): *m/z* (%): 607 (100) $[M + H]^+$, 324 (75) $[M–DKP+H]^+$; **HRMS** (ESI): calcd. for $C_{35}H_{39}N_6O_4^+$ $[M+H]^+$: 607.3027; found: 607.3021.

(3*R*,3'*R*,8a*S*,8a'*S*)-3,3'-((propane-1,1-diylbis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 200b



Using the general procedure with diketopiperazine isomer **2b** and propionaldehyde on a 1.0 mmol scale, compound **200b** was obtained after reversed-phase chromatography using a H_2O/ACN gradient (2 CVs 15% ACN, over 30 CVs to 40% ACN). **Yield** 12% (0.070 g); white foam; m.p. 188-193 °C; $[\alpha]_D^{25}$ = +135.5 (c=0.43 in CH_2Cl_2); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 0.80-1.12 (7H, m, C<u>H</u>-12, C<u>H</u>$_a$H$_b$-14, C<u>H$_2$</u>-13, C<u>H$_3$</u>-20), 1.46-1.55 (1H, m, CH$_a$<u>H$_b$</u>-14), 1.84-2.12 (3H, m, C<u>H</u>$_a$H$_b$-13', C<u>H$_2$</u>-14'), 2.37-2.58 (3H, m, CH$_a$<u>H$_b$</u>-13', C<u>H$_2$</u>-19), 2.72 (1H, ddd, *J*=12.0 Hz, *J*=9.6 Hz, *J*=2.4 Hz, C<u>H</u>$_a$H$_b$-15), 2.91 (1H, dd, *J*=14.7 Hz, *J*=5.1 Hz, C<u>H</u>$_a$H$_b$-8), 3.01 (1H, dd, *J*=14.2 Hz, *J*=11.7 Hz, C<u>H</u>$_a$H$_b$-8'), 3.26 (1H, dt, *J*=12.0 Hz, *J*=8.6 Hz, CH$_a$<u>H$_b$</u>-15), 3.51-3.62 (3H, m, CH$_a$<u>H$_b$</u>-8, CH$_a$<u>H$_b$</u>-8', C<u>H</u>$_a$H$_b$-15'), 3.67-3.76 (1H, m, CH$_a$<u>H$_b$</u>-15'), 4.01 (1H, dd, *J*=10.0 Hz, *J*=6.7 Hz, C<u>H</u>-12'), 4.13-4.17 (1H, m, C<u>H</u>-9), 4.52 (1H, ddd, *J*=11.7 Hz, *J*=4.2 Hz, *J*=4.2 Hz, C<u>H</u>-9'), 6.63 (1H, t, *J*=7.4 Hz, C<u>H</u>-18), 6.76 (1H, s, C<u>H</u>-2), 7.07-7.18 (3H, m, C<u>H</u>-5, C<u>H</u>-5', C<u>H</u>-6'), 7.21 (1H, d, *J*=7.8 Hz, C<u>H</u>-7'), 7.27 (1H, s, C<u>H</u>-2'), 7.32 (1H, ddd, *J*=7.9 Hz, *J*=7.9 Hz, *J*=1.0 Hz, C<u>H</u>-6), 7.54 (1H, d, *J*=7.9 Hz, C<u>H</u>-4), 7.62 (1H, d, *J*=7.9 Hz, C<u>H</u>-7), 7.72 (1H, d, *J*=7.8 Hz, C<u>H</u>-4'), 7.92 (1H, d, *J*=2.8 Hz, NH-10), 9.14 (1H, d, *J*=4.2 Hz, NH-10') ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 10.5 (CH_3-20), 21.0 (CH_2-14), 22.2 (CH_2-14'), 28.1 (CH_2-13), 28.8 (CH_2-19), 29.1 (CH_2-13'), 30.3 (CH_2-8), 30.7 (CH_2-8'), 44.6 (CH_2-15), 45.4 (CH_2-15'), 56.2 (CH-9'), 56.9 (CH-12), 57.4 (CH-9), 58.4 (CH-12'), 65.8 (CH-18), 108.0 (CH-7), 108.6 (CH-7'), 110.1 (C_q-3), 111.1 (C_q-3'), 19.9 (CH-4, CH-5), 120.1 (CH-4'), 120.6 (CH-5'), 122.5 (CH-6'), 122.9 (CH-6), 123.0 (CH-2'), 123.6 (CH-2), 127.2 (C_q-3a), 127.9 (C_q-3a'), 136.2 (C_q-7a), 136.8 (C_q-7a'), 165.1 ($C_{C=O}$-17), 166.2 ($C_{C=O}$-17'), 170.2 ($C_{C=O}$-11), 170.4 ($C_{C=O}$-11') ppm (The allocation of the signals CH_y-X and CH_y-X' may be interchanged); **IR** (cm^{-1}): υmax = 3219 (NH), 1645 (C=O), 1454; **MS** (ES): *m/z* (%): 607 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{35}H_{39}N_6O_4^+$ $[M+H]^+$: 607.3027; found: 607.3019.

(3*S*,3'*S*,8a*S*,8a'*S*)-3,3'-(((phenylmethylene)bis(1*H*-indole-2,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 204a

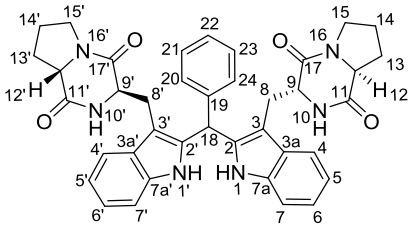


Using the general procedure with DKP isomer **2a** and benzaldehyde on a 0.7 mmol scale, compound **204a** was obtained after column chromatography. **Yield** 36% (0.165 g); orange/brown powder; R_f=0.03 (CH_2Cl_2/2% MeOH); m.p. 189-195 °C; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.57-1.89 (6H, m, C<u>H</u>$_a$H$_b$-13, C<u>H</u>$_a$H$_b$-13',C<u>H$_2$</u>-14, C<u>H$_2$</u>-14'); 2.08-2.22 (2H, m, CH$_a$<u>H$_b$</u>-13, CH$_a$<u>H$_b$</u>-13'); 2.98-3.11 (2H, m, C<u>H</u>$_a$H$_b$-8,

C$\underline{H}_a$H$_b$-8‘); 3.32-3.58 (6H, m, CH$_a\underline{H}_b$-8, CH$_a\underline{H}_b$-8‘,C$\underline{H}_2$-15, C$\underline{H}_2$-15‘); 3.68-3.82 (2H, m, C$\underline{H}$-12, C$\underline{H}$-12‘); 3.96 (1H, dd, *J*=8.0 Hz, *J*=5.2 Hz, C$\underline{H}$-9); 4.05 (1H, dd, *J*=6.6 Hz, *J*=6.6 Hz, C$\underline{H}$-9‘); 5.87 (1H, s, N$\underline{H}$-10); 6.17 (1H, s, N$\underline{H}$ 10‘); 6.34 (1H, s, C$\underline{H}$-18); 7.11-7.38 (11H, m, C$\underline{H}$-5, C$\underline{H}$-5‘,C$\underline{H}$-6, C$\underline{H}$-6‘,C$\underline{H}$-7, C$\underline{H}$-7‘,C$\underline{H}$-20, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24); 7.51-7.58 (2H, m, C$\underline{H}$-4, C$\underline{H}$-4‘); 8.28 (2H, s, N$\underline{H}$-1, N$\underline{H}$-1‘) ppm; **^{13}C-NMR (75 MHz, CDCl$_3$) δ** 22.4 (CH$_2$-14); 22.6 (CH$_2$-14‘); 25.7 (CH$_2$-8); 25.9 (CH$_2$-8‘); 28.1 (CH$_2$-13); 28.1 (CH$_2$-13‘); 41.1 (CH-18); 45.2 (CH$_2$-15); 45.4 (CH$_2$-15‘); 54.7 (CH-9); 55.2 (CH-9‘); 59.1 (CH-12, CH-12‘); 106.9 (C$_q$-3); 107.2 (C$_q$-3‘); 111.5 (CH-7); 111.6 (CH-7‘); 118.3 (CH-4); 118.6 (CH-4‘); 120.5 (CH-5, CH-5‘); 122.6 (CH-6, CH-6‘); 128.0 (CH-22); 128.6 (CH-21, CH-23); 128.7 (C$_q$-3a); 128.8 (C$_q$-3a‘); 129.5 (CH-20, CH-24); 135.3 (C$_q$-2*); 135.5 (C$_q$-2‘*); 135.6 (C$_q$-7a*); 136.0 (C$_q$-7a‘*); 139.8 (C$_q$-19); 165.6 (C$_{C=O}$-11**); 165.8 (C$_{C=O}$-11‘**); 169.2 (C$_{C=O}$-17**); 169.7 (C$_{C=O}$-17‘**) ppm (The allocation of the signals CH$_y$-X and CH$_y$-X‘ as well as signals with the same superscript (*, **) may be interchanged); IR (cm^{-1}): υmax = 3252 (NH), 1655 (C=O), 1457, 1432; MS (ES): *m/z* (%): 655 (100) [M + H]$^+$, 327 (70) [M–DKP+H]$^+$; HRMS (ESI): calcd. for $C_{39}H_{39}N_6O_4^+$ [M+H]$^+$: 655.3033; found: 655.3019.

Using the general procedure with DKP isomer **2b** and benzaldehyde on a 2.5 mmol scale, compounds **204b** and **205** were obtained after pHPLC using an isocratic H$_2$O/ACN gradient (35% ACN).

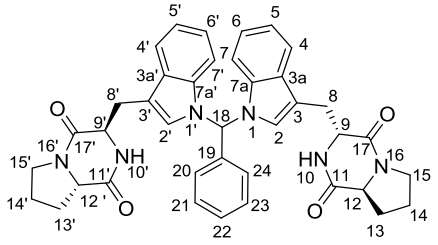
(3*R*,3'*R*,8a*S*,8a'*S*)-3,3'-(((phenylmethylene)bis(1*H*-indole-2,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 204b



Yield 8% (0.013 g); white amorphous powder; m.p. 150-154 °C; **^{1}H-NMR (400 MHz, CDCl$_3$) δ** 1.24-1.39 (2H, m, C$\underline{H}_a$H$_b$-14, C$\underline{H}_a$H$_b$-14’), 1.60-1.84 (4H, m, C$\underline{H}_a$H$_b$-13, C$\underline{H}_a$H$_b$-13’, CH$_a\underline{H}_b$-14, CH$_a\underline{H}_b$-14’), 1.96-2.09 (2H, m, CH$_a\underline{H}_b$-13, CH$_a\underline{H}_b$-13’), 2.65-2.81 (3H, m, C$\underline{H}_a$H$_b$-8, C$\underline{H}$-12, C$\underline{H}$-12’), 2.83 (1H, dd, *J*=14.6 Hz, *J*=5.0 Hz, C$\underline{H}_a$H$_b$-8’), 3.01 (1H, dd, *J*=10.0 Hz, *J*=10.0 Hz, C$\underline{H}_a$H$_b$-15’), 3.12-3.23 (2H, m, CH$_a\underline{H}_b$-8, CH$_a\underline{H}_b$-15’), 3.25-3.35 (1H, m, CH$_a\underline{H}_b$-8’), 3.37-3.49 (2H, m, C$\underline{H}_2$-15), 3.93 (1H, br. s, C$\underline{H}$-9), 4.16-4.23 (1H, m, C$\underline{H}$-9’), 6.05 (1H, s, C$\underline{H}$-18), 7.03-7.33 (11H, m, C$\underline{H}$-5, C$\underline{H}$-5’, C$\underline{H}$-6, C$\underline{H}$-6’, C$\underline{H}$-7, C$\underline{H}$-7’, C$\underline{H}$-20, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24), 7.45-7.54 (2H, m, C$\underline{H}$-4, C$\underline{H}$-4’), 7.68 (1H, s, N$\underline{H}$-10’), 8.09 (1H, s, N$\underline{H}$-1’), 8.16 (1H, s, N$\underline{H}$-10), 8.36 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (100 MHz, CDCl$_3$) δ** 21.5 (CH$_2$-14), 21.6 (CH$_2$-14’), 28.9 (CH$_2$-13, CH$_2$-13’), 29.3 (CH$_2$-8), 29.4 (CH$_2$-8’), 41.9 (CH-18), 44.9 (CH$_2$-15), 45.1 (CH$_2$-15’), 57.9 (CH-12, CH-12’), 58.1 (CH-9’), 58.2 (CH-9), 106.6 (C$_q$-3), 107.3 (C$_q$-3’), 111.1 (CH-7), 111.3 (CH-7’), 118.6 (CH-4), 118.9 (CH-4’), 120.1 (CH-5, CH-5’), 122.1 (CH-6), 122.4 (CH-6’), 128.0 (CH-22), 128.5 (C$_q$-3a), 128.9 (CH-20, CH-24), 129.3 (C$_q$-3a’), 129.4 (CH-21, CH-23), 134.6 (C$_q$-7a), 134.9 (C$_q$-2), 135.1 (C$_q$-7a’), 135.8 (C$_q$-2), 139.3 (C$_q$-19), 165.9 (C$_{C=O}$-17*, C$_{C=O}$-17’*), 169.9 (C$_{C=O}$-11*),

170.2 ($C_{C=O}$-11'*) ppm (The allocation of the signals CH_y-X and CH_y-X' as well as signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 3250 (NH), 1651 (C=O), 1458; **MS** (ES): *m/z* (%): 655 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{39}H_{39}N_6O_4^+$ $[M+H]^+$: 655.3027; found: 655.3036.

(3*R*,3'*R*,8a*S*,8a'*S*)-3,3'-(((phenylmethylene)bis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione) 205



Yield 11% (0.015 g); white amorphous powder; m.p. 190-196 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 0.89-1.12 (2H, m, C$\underline{H}_a$$H_b$-13, C$\underline{H}_a$$H_b$-14), 1.18-1.27 (2H, m, C$\underline{H}$-12, $CH_a\underline{H}_b$-13), 1.51-1.60 (1H, m, $CH_a\underline{H}_b$-14), 1.78-2.10 (3H, m, C$\underline{H}_a$$H_b$-13', C$\underline{H}_2$-14'), 2.33-2.41 (1H, m, $CH_a\underline{H}_b$-13'), 2.67-2.75 (1H, m, C$\underline{H}_a$$H_b$-15), 2.86-2.98 (2H, m, C$\underline{H}_a$$H_b$-8, C$\underline{H}_a$$H_b$-8'), 3.30 (1H, dt, *J*=12.1 Hz, *J*=8.6 Hz, $CH_a\underline{H}_b$-15'), 3.50-3.74 (4H, m, $CH_a\underline{H}_b$-8, $CH_a\underline{H}_b$-8', C$\underline{H}_2$-15'), 3.99 (1H, dd, *J*=10.0 Hz, *J*=6.6 Hz, C$\underline{H}$-12'), 4.16-4.21 (1H, m, C$\underline{H}$-9), 4.56 (1H, ddd, *J*=11.8 Hz, *J*=4.2 Hz, *J*=4.2 Hz, C$\underline{H}$-9'), 6.71 (1H, s, C$\underline{H}$-2), 6.81 (1H, s, C$\underline{H}$-2'), 7.00 (2H, d, *J*=6.9 Hz, C$\underline{H}$-20, C$\underline{H}$-24), 7.14-7.25 (3H, m, C$\underline{H}$-5, C$\underline{H}$-5', C$\underline{H}$-6), 7.27-7.43 (5H, m, C$\underline{H}$-6', C$\underline{H}$-7', C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23), 7.54 (1H, d, *J*=8.0 Hz, C$\underline{H}$-7), 7.63 (1H, d, *J*=8.0 Hz, C$\underline{H}$-4'), 7.77 (1H, d, *J*=8.0 Hz, C$\underline{H}$-4), 8.04 (1H, s, C$\underline{H}$-18), 8.12 (1H, d, *J*=2.7 Hz, N$\underline{H}$-10), 8.12 (1H, d, *J*=4.2 Hz, N$\underline{H}$-10') ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 21.0 (CH_2-14), 22.2 (CH_2-14'), 28.2 (CH_2-13), 29.0 (CH_2-13'), 30.3 (CH_2-8), 30.7 (CH_2-8'), 44.5 (CH_2-15), 45.4 (CH_2-15'), 56.1 (CH-9'), 57.1 (CH-12), 57.4 (CH-9), 58.3 (CH-12'), 66.5 (CH-18), 108.2 (CH-7), 108.4 (CH-7'), 110.2 (C_q-3), 111.4 (C_q-3'), 119.9 (CH-4'), 120.4 (CH-4, CH-5), 121.0 (CH-5'), 122.7 (CH-6), 123.2 (CH-6'), 124.9 (CH-2), 125.4 (CH-2'), 127.1 (CH-20, CH-24), 127.4 (C_q-3a), 128.1 (C_q-3a'), 129.1 (CH-21, CH-23), 129.3 (CH-22), 136.2 (C_q-7a), 136.4 (C_q-19), 137.4 (C_q-7a'), 165.0 ($C_{C=O}$-17), 166.2 ($C_{C=O}$-17'), 170.3 ($C_{C=O}$-11), 170.4 ($C_{C=O}$-11') ppm (The allocation of the signals CH_y-X and CH_y-X' may be interchanged); **IR** (cm^{-1}): υmax = 3219 (NH), 1645 (C=O), 1454; **MS** (ES): *m/z* (%): 655 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{39}H_{39}N_6O_4^+$ $[M+H]^+$: 655.3027; found: 655.3040.

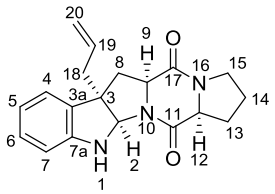
6. Ring formation through metathesis of allyl groups

6.1. Introduction of the allyl group at C-3 of the indole[189]

Diketopiperazine **2** (1 equiv., 0.35 mmol, 0.10 g) and $Pd(PPh_3)_4$ (5 mol%, 0.018 mmol, 0.020 g) were dissolved in dry, degassed THF (5 mL) at room temperature under a nitrogen atmosphere. Allyl alcohol (1 equiv., 0.35 mmol, 0.024 mL) and Et_3B (1 equiv., 0.35 mmol, 0.35 mL of 1 M hexane solution,) were successively added to this solution. The reaction mixture was stirred at 50 °C for 5 hours, during which the reaction was monitored by means of HPLC-MS. After dilution with EtOAc

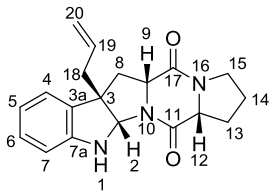
(5 mL), the mixture was washed twice with saturated aq. $NaHCO_3$ (5 mL) and with brine (5 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo.* The residue was subjected to reversed-phase pHPLC using an isocratic H_2O/ACN gradient (35% ACN) to yield the desired compounds **222**.

(5a*S*,6a*R*,11a*R*,13a*S*)-6a-allyl-1,2,3,6,6a,11,11a,13a-octahydro-13*H*-pyrrolo[1'',2'':4',5']pyrazino-[1',2':1,5]pyrrolo[2,3-b]indole-5,13(5a*H*)-dione 222a



Following the general procedure using isomer **2a**, compound **222a** was obtained after purification by pHPLC. **Yield** 76% (0.086 g); colourless crystals; m.p. 85-87 °C; $[\alpha]_D^{25}$ = -302.8 (c=0.27 in $CHCl_3$); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.63 (1H, br s, NH-1), 1.83-1.97 (1H, m, CH_aH_b-14), 1.97-2.09 (1H, m, CH_aH_b-14), 2.09-2.20 (1H, m, CH_aH_b-13), 2.26-2.35 (1H, m, CH_aH_b-13), 2.36 (1H, dd, *J*=13.0 Hz, *J*=11.1 Hz, CH_aH_b-8), 2.45-2.49 (2H, m, CH_2-18), 2.67 (1H, dd, *J*=13.0 Hz, *J*=6.3 Hz, CH_aH_b-8), 3.44-3.60 (2H, m, CH_2-15), 4.04-4.11 (2H, m, CH-9, CH-12), 5.06-5.10 (1H, m, CH_aH_b-20), 5.11 (1H, br s, CH_aH_b-20), 5.30 (1H, s, CH-2), 5.65-5.80 (1H, m, CH-19), 6.62 (1H, d, *J*=7.5 Hz, CH-7), 6.79 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, CH-5), 7.07-7.13 (2H, m, CH-4, CH-6) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 23.1 (CH_2-14), 27.7 (CH_2-13), 37.8 (CH_2-8), 41.9 (CH_2-18), 45.2 (CH_2-15), 55.5 (C_q-3), 60.3 (CH-9*), 60.5 (CH-12*), 79.2 (CH-2), 109.6 (CH-7), 119.1 (CH_2-20), 119.4 (CH-5), 123.5 (CH-4), 128.8 (CH-6), 130.8 (C_q-3a), 132.9 (CH-19), 148.9 (C_q-7a), 165.9 ($C_{C=O}$-17), 167.1 ($C_{C=O}$-11) ppm; IR (cm^{-1}): υmax = 1665 (C=O), 1632 (C=O), 1426; MS (ES): *m/z* (%): 324 (100) $[M + H]^+$; HRMS (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M+H]^+$: 324.1707; found: 324.1703.

(5a*R*,6a*S*,11a*S*,13a*R*)-6a-allyl-1,2,3,6,6a,11,11a,13a-octahydro-13*H*-pyrrolo[1'',2'':4',5']pyrazino-[1',2':1,5]pyrrolo[2,3-b]indole-5,13(5a*H*)-dione 222b



Following the general procedure using isomer **2d**,compound **222b** was obtained after purification by pHPLC. **Yield** 68% (0.077 g); colourless crystals; m.p. 85-87 °C; $[\alpha]_D^{25}$ = +302.5 (c=0.27 in $CHCl_3$); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.78-1.96 (2H, m, NH-1, CH_aH_b-14), 1.96- 2.08 (1H, m, CH_aH_b-14), 2.09-2.20 (1H, m, CH_aH_b-13), 2.26-2.34 (1H, m, CH_aH_b-13), 2.36 (1H, dd, *J*=12.6 Hz, *J*=11.9 Hz, CH_aH_b-8), 2.45-2.55 (2H, m, CH_2-18), 2.66 (1H, dd, *J*=12.6 Hz, *J*=6.3 Hz, CH_aH_b-8), 3.41-3.60 (2H, m, CH_2-15), 4.04-4.10 (1H, m, CH-9, CH-12), 5.05-5.15 (2H, m, CH_2-20), 5.30 (1H, s, CH-2), 5.65-5.80 (1H, m, CH-19), 6.61 (1H, d, *J*=7.5 Hz, CH-7), 6.79 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, CH-5), 7.07-7.14 (2H, m, CH-4, CH-6); **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 23.1 (CH_2-14), 27.7 (CH_2-13), 37.8 (CH_2-8), 41.9 (CH_2-18), 45.2 (CH_2-15), 55.5 (C_q-3), 60.3 (CH-9*), 60.5 (CH-12*), 79.2 (CH-2), 109.5 (CH-7), 119.1 (CH_2-20), 119.4 (CH-5),

123.5 (CH-4), 128.8 (CH-6), 130.8 (C_q-3a), 132.9 (CH-19), 148.9 (C_q-7a), 165.9 ($C_{C=O}$-17), 167.1 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 1666 (C=O), 1632 (C=O), 1425; **MS** (ES): *m/z* (%): 324 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M+H]^+$: 324.1707; found: 324.1708.

6.2. Selective synthesis of mono-allylated diketopiperazine 228a

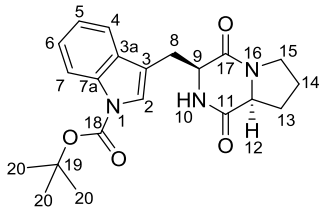
6.2.1. Synthesis of N'-Boc-protected dipeptide 229

Crude dipeptide **181a** (1 equiv., 1.0 mmol, 0.45 g) synthesized according to the general procedure in 4.1.1 was dissolved in dry CH_2Cl_2 (20 mL) under a nitrogen atmosphere. Boc_2O (1 equiv., 1.0 mmol, 0.22 g), triethylamine (2 equiv., 2.0 mmol, 0.28 mL) and DMAP (0.2 equiv, 0.20 mmol, 24 mg) were added to the solution. After 4 hours the reaction mixture was washed three times with brine (15 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo* yielding the *N*'-Boc-protected dipeptide **229** (quant., 1.0 mmol, 0.55 g) as a yellow foam.

6.2.2. Hydrogenolysis and cyclization

To a solution of *N*'-Boc-protected dipeptide **229** (1 equiv., 1.0 mmol, 0.55 g) in MeOH (25 mL), 10 wt% of Pd/C (0.055 g) was added. The reaction mixture was stirred under 5 atm of H_2 for 24 hours at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. Next, the solution was transferred in a closed vessel and ammonia in methanol (7 M NH_3) was added to induce ring formation. The filtrate was concentrated *in vacuo* to give the crude *N*'-Boc-protected diketopiperazine **230**. The pure product **230** was obtained after column chromatography with EtOAc as eluent.

tert-butyl 3-(((3*S*,8a*S*)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indole-1-carboxylate 230



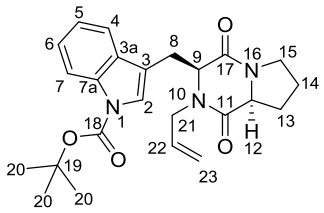
Following the general procedures 6.2.1 and 6.2.2 afforded compound **230**. **Yield** 92% (0.35 g); colourless crystals; R_f=0.17 (EtOAc); m.p. 143-145 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.69 (9H, s, 3×C<u>H</u>$_3$-20), 1.83-1.99 (1H, m, C<u>H</u>$_a$H$_b$-14), 2.00-2.13 (2H, m, C<u>H</u>$_a$H$_b$-13, CH$_a$<u>H</u>$_b$-14), 2.29-2.40 (1H, m, CH$_a$<u>H</u>$_b$-13), 2.90 (1H, dd, *J*=15.1 Hz, *J*=11.0 Hz, C<u>H</u>$_a$H$_b$-8), 3.55-3.69 (2H, m, C<u>H</u>$_2$-15), 3.72 (1H, dd, *J*=15.1 Hz, *J*=2.8 Hz, CH$_a$<u>H</u>$_b$-8), 4.06-4.13 (1H, m, C<u>H</u>-12), 4.38 (1H, dd, *J*=11.0 Hz , *J*=2.8 Hz, C<u>H</u>-9), 5.77 (1H, s, N<u>H</u>-10), 7.26 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C<u>H</u>-5), 7.36 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C<u>H</u>-6), 7.51 (1H, s, C<u>H</u>-2), 7.52 (1H, d, *J*=7.8 Hz, C<u>H</u>-4), 8.17 (1H, d, *J*=7.8 Hz, C<u>H</u>-7) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 22.7 (CH_2-14), 26.6 (CH_2-8), 28.2 (3×CH_3-20), 28.3 (CH_2-13), 45.5 (CH_2-15), 54.0 (CH-9), 59.2 (CH-12), 84.1 (C_q-19), 114.8 (C_q-3), 115.7 (CH-7), 118.7 (CH-4), 122.9 (CH-5), 124.5 (CH-2), 125.1 (CH-6), 129.4 (C_q-3a), 135.9 (C_q-7a), 149.3 ($C_{C=O}$-18), 165.1 ($C_{C=O}$-17), 169.4

($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 1736 (C=O), 1645 (C=O), 1370; **MS** (ES): *m/z* (%): 384 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{21}H_{26}N_3O_4^+$ $[M+H]^+$: 384.1918; found: 384.1912.

6.2.3. Allylation

To a solution of *N*'-Boc-protected diketopiperazine **230** (1 equiv., 1.3 mmol, 0.50 g) in dry ACN (25 mL), sodium hydride was added in two separate portions (one portion every 15 minutes). Subsequently, two equivalents of allyl bromide (2 equiv., 2.6 mmol, 0.23 mL) were added. The mixture was stirred under a nitrogen atmosphere for 16 hours at room temperature. Then, the reaction was quenched by the addition of water and the solvent was evaporated under reduced pressure. Subsequently, the residue was dissolved in CH_2Cl_2 (30 mL). The solution was washed twice with saturated aq. $NaHCO_3$ (30 mL). The organic phase was dried over magnesium sulfate and concentrated *in vacuo*. Purification of the residue by chromatography provided the desired product **231**.

tert-butyl 3-(((3*S*,8a*S*)-2-allyl-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indole-1-carboxylate 231

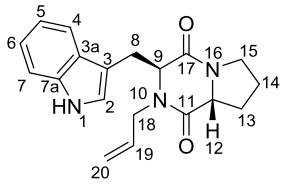


Yield 87% (0.48 g); colourless crystals; R_f=0.25 (1/3 petroleum ether/EtOAc); m.p. 147-149 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 0.45-0.57 (1H, m, C$\underline{H_a}H_b$-13), 1.15-1.26 (1H, m, C$\underline{H_a}H_b$-14), 1.48-1.60 (1H, m, $CH_a\underline{H_b}$-14), 1.66 (9H, s, 3×C$\underline{H_3}$-20), 1.94-2.02 (1H, m, $CH_a\underline{H_b}$-13), 3.13 (1H, ddd, *J*=12.0 Hz, *J*=10.1 Hz, *J*= 4.5 Hz, C$\underline{H_a}H_b$-15), 3.26 (1H, dd, *J*=15.4 Hz, *J*=3.9 Hz, C$\underline{H_a}H_b$-8), 3.51-3.63 (3H, m, $CH_a\underline{H_b}$-8, C$\underline{H_a}H_b$-21, $CH_a\underline{H_b}$-15), 3.83 (1H, dd, *J*=11.3 Hz, *J*=6.2 Hz, C$\underline{H}$-12), 4.41 (1H, dd, *J*=3.9 Hz, *J*=3.9 Hz, C$\underline{H}$-9), 4.90 (1H, dddd, *J*=15.3 Hz, *J*=4.6 Hz, *J*=1.7 Hz, *J*=1.7 Hz, $CH_a\underline{H_b}$-21), 5.29-5.35 (2H, m, C$\underline{H_2}$-23), 5.76-5.87 (1H, m, C$\underline{H}$-22), 7.23 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.1 Hz, C$\underline{H}$-5), 7.28-7.31 (2H, m, C$\underline{H}$-2, C$\underline{H}$-6), 7.55 (1H, dd, *J*=7.7 Hz, *J*=1.1 Hz, C$\underline{H}$-4), 8.09 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 21.1 (CH_2-14), 25.9 (CH_2-8), 28.2 (3×CH_3-20), 28.9 (CH_2-13), 44.6 (CH_2-15), 45.5 (CH_2-21), 59.1 (CH-12), 59.3 (CH-9), 83.9 (C_q-19), 113.9 (C_q-3), 115.1 (CH-7), 119.2 (CH_2-23), 119.4 (CH-4), 122.6 (CH-5), 124.7 (CH-2*), 124.8 (CH-6*), 130.2 (C_q-3a), 131.8 (CH-22), 135.1 (C_q-7a), 149.4 ($C_{C=O}$-18), 164.1 ($C_{C=O}$-17), 165.9 ($C_{C=O}$-11) ppm (The signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 1742 (C=O), 1644 (C=O), 1632 (C=O), 1365; **MS** (ES): *m/z* (%): 424 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M - Boc + 2H]^+$: 324.1707; found: 324.1703.

6.2.4. Boc deprotection

Acetyl chloride (5 equiv., 15.5 mmol, 1.1 mL) was added dropwise to a solution of compound **231** (1 equiv., 3.1 mmol, 1.3 g) in 25 mL of methanol at 0 °C. After stirring at room temperature for 24 hours the mixture was concentrated under reduced pressure. The mono-allylated product **228a** was obtained through purification by column chromatography using a mixture of EtAOc and petroleum ether as eluent.

(3*S*,8a*R*)-3-((1*H*-indol-3-yl)methyl)-2-allylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione 228a

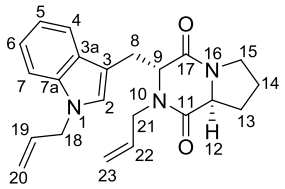


Yield 82% (0.81 g); colourless crystals; R_f=0.23 (1/4 petroleum ether/EtOAc); m.p. 153-155 °C; $[\alpha]_D^{25}$ = +75.3 (c=0.59 in $CHCl_3$); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.11-1.28 (1H, m, C$\underline{H_a}$H$_b$-14), 1.55-1.67 (1H, m, C$\underline{H_a}$H$_b$-13), 1.67-1.78 (1 H, m, CH$_a\underline{H_b}$-14), 1.88-1.96 (1H, m, CH$_a\underline{H_b}$-13), 2.29 (1H, dd, *J*=10.8 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 3.03 (1H, ddd, *J*=11.8 Hz, *J*=9.7 Hz, *J*=2.2 Hz, C$\underline{H_a}$H$_b$-15), 3.24 (1H, dd, *J*=14.9 Hz, *J*=4.5 Hz, C$\underline{H_a}$H$_b$-8), 3.41-3.54 (3H, m, CH$_a\underline{H_b}$-8, CH$_a\underline{H_b}$-15, C$\underline{H_a}$H$_b$-18), 4.27 (1H, dd, *J*=4.5 Hz, *J*=4.5 Hz , C$\underline{H}$-9), 4.67 (1H, dd, *J*=15.0 Hz, *J*=5.0 Hz, CH$_a\underline{H_b}$-18), 5.17-5.23 (2H, m, C$\underline{H_2}$-20), 5.78 (1H, dddd, *J*=17.0 Hz, *J*=10.0 Hz, *J*=7.2 Hz, *J*=5.0 Hz, C$\underline{H}$-19), 6.92 (1H, d, *J*=2.1 Hz, C$\underline{H}$-2), 7.09 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-5), 7.16 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-6), 7.33 (1H, d, *J*=7.6 Hz, C$\underline{H}$-7), 7.57 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4), 9.04 (1H, br s, N$\underline{H}$-1) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 21.6 (CH_2-14), 27.1 (CH_2-8), 29.2 (CH_2-13), 44.9 (CH_2-15), 46.5 (CH_2-18), 57.9 (CH-12), 62.1 (CH-9), 108.9 (C_q-3), 111.4 (CH-7), 118.8 (CH-4), 119.1 (CH_2-20), 119.6 (CH-5), 122.3 (CH-6), 124.2 (CH-2), 127.2 (C_q-3a), 131.9 (CH-19), 136.2 (C_q-7a), 165.5 ($C_{C=O}$-17), 167.8 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 1739 (C=O), 1455; **MS** (ES): *m/z* (%): 324 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M + H]^+$: 324.1707; found: 324.1712.

6.3. Synthesis of diallylated diketopiperazines[121]

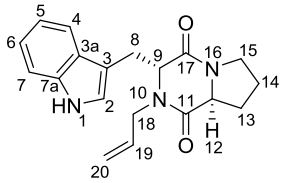
To a stirred solution of cyclo(D-Trp, L-Pro) **2b** (1 equiv., 1.1 mmol, 300 mg) in 10 mL of dry DMF, sodium hydride (2.2 equiv., 2.3 mmol, 56 mg) was added at 0 °C. After stirring the mixture at 0 °C for 15 min, allyl bromide (5 equiv., 5.3 mmol, 0.5 mL) was added. The reaction mixture was stirred at 0 °C for another hour and was then allowed to warm to room temperature. The reaction was monitored using HPLC-MS. When the conversion stagnated, an extra portion of sodium hydride was added (1 equiv., 1.1 mmol, 25 mg). The reaction was then quenched with 5% aq. $NaHCO_3$ (30 mL) and extracted two times with CH_2Cl_2 (2×25 mL). The combined organic phases were washed with brine (2×25 mL), and dried over magnesium sulfate. Removal of the solvent under reduced pressure gave the crude reaction product containing mono- and diallylated products, which were separated by means of column chromatography (1/1 petroleum ether/EtOAc).

(3*R*,8a*S*)-2-allyl-3-((1-allyl-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione 220b



Yield 43% (0.165 g); yellow oil; R_f=0.27 (1/1 petroleum ether/EtOAc); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.13-1.38 (1H, m, C$\underline{H_a}H_b$-14), 1.54-1.82 (2H, m, C$\underline{H_a}H_b$-13, $CH_a\underline{H_b}$-14), 1.90-2.00 (1H, m, $CH_a\underline{H_b}$-13), 2.35 (1H, dd, *J*=10.7 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 3.00-3.10 (1H, m, C$\underline{H_a}H_b$-15), 3.23 (1H, dd, *J*=14.9 Hz, *J*=4.6 Hz, C$\underline{H_a}H_b$-8), 3.39-3.53 (3H, m, $CH_a\underline{H_b}$-8, $CH_a\underline{H_b}$-15, C$\underline{H_a}H_b$-21), 4.25 (1H, t, *J*=4.6 Hz, C$\underline{H}$-9), 4.62-4.72 (3H, m, C$\underline{H_2}$-18, $CH_a\underline{H_b}$-21), 5.05 (1H, ddd, *J*=17.1 Hz, *J*=2.8 Hz, *J*=1.7 Hz, C$\underline{H_a}H_b$-20), 5.14-5.28 (3H, m, $CH_a\underline{H_b}$-20, C$\underline{H_2}$-23), 5.76 (1H, dddd, *J*=17.1 Hz, *J*=9.8 Hz, *J*=7.5 Hz, *J*=5.0 Hz, C$\underline{H}$-22), 5.92 (1H, dddd, *J*=17.1 Hz, *J*=10.5 Hz, *J*=5.3 Hz, *J*=5.2 Hz, C$\underline{H}$-19), 6.87 (1H, s, C$\underline{H}$-2), 7.10 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.18 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-6), 7.27 (1H, d, *J*=7.5 Hz, C$\underline{H}$-7), 7.56 (1H, d, *J*=7.5 Hz, C$\underline{H}$-4) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.7 (CH_2-14), 27.0 (CH_2-8), 29.1 (CH_2-13), 44.9 (CH_2-15), 46.4 (CH_2-21), 48.7 (CH_2-18), 57.9 (CH-12), 62.1 (CH-9), 108.3 (C_q-3), 109.6 (CH-7), 117.4 (CH_2-20), 119.0 (CH_2-23), 119.1 (CH-4), 119.5 (CH-5), 122.2 (CH-6), 127.6 (CH-2), 128.0 (C_q-3a), 132.0 (CH-22), 133.3 (CH-19), 136.3 (C_q-7a), 165.6 ($C_{C=O}$-17), 167.4 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 1651 (C=O), 1451; **MS** (ES): *m/z* (%): 364 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{22}H_{26}N_3O_2^+$ $[M + H]^+$: 364.1707; found: 364.2038.

(3*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-2-allylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione 228b



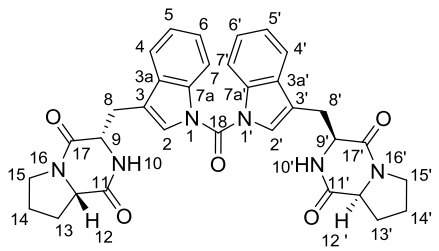
Yield 13% (0.044 g); colourless crystals; R_f=0.20 (1/1 petroleum ether/EtOAc); m.p. 163-165 °C; $[\alpha]_D^{25}$ = -95.2 (c=0.59 in $CHCl_3$); **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.10-1.34 (1H, m, C$\underline{H_a}H_b$-14), 1.54-1.79 (2H, m, C$\underline{H_a}H_b$-13, $CH_a\underline{H_b}$-14), 1.86-1.98 (1H, m, $CH_a\underline{H_b}$-13), 2.29 (1H, dd, *J*=10.7 Hz, *J*=6.3 Hz, C$\underline{H}$-12), 2.97-3.09 (1H, m, C$\underline{H_a}H_b$-15), 3.24 (1H, dd, *J*=14.9 Hz, *J*=4.5 Hz, C$\underline{H_a}H_b$-8), 3.40-3.55 (3H, m, $CH_a\underline{H_b}$-8, $CH_a\underline{H_b}$-15, C$\underline{H_a}H_b$-18), 4.27 (1H, dd, *J*=4.5 Hz, *J*=4.5 Hz, C$\underline{H}$-9), 4.68 (1H, dd, *J*=15.7 Hz, *J*=5.0 Hz, $CH_a\underline{H_b}$-18), 5.21 (1H, d, *J*=16.9 Hz, C$\underline{H_a}H_b$-20), 5.25 (1H, d, *J*=10.1 Hz, $CH_a\underline{H_b}$-20), 5.76 (1H, dddd, *J*=16.9 Hz, *J*=10.1 Hz, *J*=7.6 Hz, *J*=5.0 Hz, C$\underline{H}$-19), 6.92 (1H, s, C$\underline{H}$-2), 7.09 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.16 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.33 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.57 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 9.02 (1H, s, N$\underline{H}$-1) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.8 (CH_2-14), 27.3 (CH_2-8), 29.5 (CH_2-13), 45.1 (CH_2-15), 46.7 (CH_2-18), 58.1 (CH-12), 62.3 (CH-9), 109.1 (C_q-3), 111.6 (CH-7), 119.0 (CH-4), 119.3 (CH_2-20), 119.9 (CH-5), 122.6 (CH-6), 124.4 (CH-2), 127.5 (C_q-3a), 132.1 (CH-19), 136.4 (C_q-7a), 165.8 ($C_{C=O}$-17), 168.0 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 1657 (C=O), 1650 (C=O), 1456; **MS** (ES): *m/z* (%): 324 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M + H]^+$: 324.1707; found: 324.1738.

7. Synthesis of annulated analogues via other electrophiles

7.1. Synthesis of a dimer with CDI

Diketopiperazine **2b** (1 equiv., 1.4 mmol, 0.40 g) was suspended in dry CH_2Cl_2 (20 mL) under a nitrogen atmosphere. Triethylamine (3 equiv., 4.2 mmol, 0.59 mL) was added and stirred for 10 minutes before the addition of CDI (1.1 equiv., 1.5 mmol, 0.25 g). The mixture was refluxed for 5 days. Subsequently, the organic phase was washed with 2 M HCl (3×30mL) and with water (3×30mL). The organic phase was dried over magnesium sulfate and concentrated *in vacuo*. Purification of the residue by pTLC provided the dimeric product **234**.

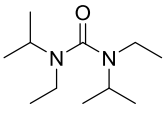
(3*S*,3'*S*,8a*S*,8a'*S*)-3,3'-((carbonylbis(1*H*-indole-1,3-diyl))bis(methylene))bis(hexahydropyrrolo[1,2-a]-pyrazine-1,4-dione) 234



Yield 65% (0.27 g); yellow foam; R_f=0.14 (CH_2Cl_2/4% MeOH); m.p. 194-196 °C; **^{1}H-NMR (300 MHz, $CDCl_3$) δ** 1.28-1.47 (2H, m, CH_aH_b-14, CH_aH_b-14‘), 1.59-1.76 (2H, m, CH_aH_b-13, CH_aH_b-13‘), 1.77-1.94 (2H, m, CH_aH_b-14, CH_aH_b-14‘), 2.06-2.17 (2H, m, CH_aH_b-13, CH_aH_b-13‘), 2.65 (2H, dd, *J*=11.6 Hz, *J*=6.1 Hz, CH-12, CH-12‘), 2.96-3.05 (2H, m, CH_aH_b-15, CH_aH_b-15‘), 3.10 (2H, dd, *J*=14.3 Hz, *J*=5.5 Hz, CH_aH_b-8, CH_aH_b-8‘), 3.71-3.57 (4H, m, CH_aH_b-8, CH_aH_b-8‘, CH_aH_b-15, CH_aH_b-15‘), 4.42-4.48 (2H, m, CH-9, CH-9‘), 7.13 (2H, s, CH-2, CH-2‘), 7.32 (2H, dd, *J*=7.8 Hz, *J*=7.8 Hz, CH-5, CH-5‘), 7.39 (2H, dd, *J*=7.8 Hz, *J*=7.8 Hz, CH-6, CH-6‘), 7.63 (2H, d, *J*=7.8 Hz, CH-4, CH-4‘), 8.10 (2H, d, *J*=7.8 Hz, CH-7, CH-7‘), 8.55 (2H, d, *J*=7.8 Hz, NH-10, NH-10‘) ppm; **^{13}C-NMR (75 MHz, $CDCl_3$) δ** 21.0 (CH_2-14, CH_2-14‘), 29.3 (CH_2-13, CH_2-13), 29.5 (CH_2-8, CH_2-8‘), 44.8 (CH_2-15, CH_2-15‘), 57.3 (CH-9, CH-9‘), 58.3 (CH-12, CH-12‘), 114.7 (CH-7, CH-7‘), 115.0 (C_q-3, C_q-3‘), 119.5 (CH-4, CH-4‘), 123.9 (CH-5, CH-5‘), 125.3 (CH-6, CH-6‘), 127.7 (CH-2, CH-2‘), 129.7 (C_q-3a, C_q-3a‘), 136.3 (C_q-7a, C_q-7a‘), 148.0 ($C_{C=O}$-18), 164.7 ($C_{C=O}$-17, $C_{C=O}$-17‘), 171.0 ($C_{C=O}$-11, $C_{C=O}$-11‘) ppm (The allocation of the signals CH_y-X and CH_y-X‘ may be interchanged); **IR** (cm^{-1}): υmax = 1713 (C=O), 1645 (C=O), 1447; **MS** (ES): *m/z* (%): 593 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{33}H_{33}N_6O_5^+$ $[M + H]^+$: 593.2507; found: 593.2499.

7.2. Synthesis of 3,5-bridged α-chloroamines 237a-d

1,3-diethyl-1,3-diisopropylurea



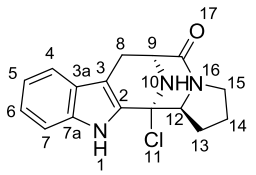
DIPEA (2 equiv., 4.0 mmol, 0.72 mL) was dissolved in dry CH_2Cl_2 (10 mL) and diphosgene (1 equiv., 2.0 mmol, 0.24 mL) was slowly added at room temperature as the reaction is exothermic. After 3 hours, the organic phase was washed with saturated aq. $NaHCO_3$ (3×5 mL), brine (2×5 mL) and with water (2×5 mL). The organic phase was

dried over magnesium sulfate and concentrated under reduced pressure yielding a yellow liquid. ^{1}H NMR revealed the presence of two rotamers in a 1:1.6 ratio.

Yield 70% (0.28 g); yellow liquid; **^{1}H NMR (400 MHz, $CDCl_3$) δ** (rotamer 1) 1.18-1.31 (18H, m, 6×C<u>H</u>$_3$), 3.32 (4H, q, *J*=6.91 Hz, 2×C<u>H</u>$_2$), 4.54 (2H, septet, *J*=6.18 Hz, 2×C<u>H</u>) ppm; (rotamer 2) 1.18-1.31 (18H, m, 6×C<u>H</u>$_3$), 3.41 (4H, q, *J*=7.06 Hz, 2×C<u>H</u>$_2$), 4.29 (2H, septet, *J*=6.55 Hz, 2×C<u>H</u>) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** (rotamer 1) 14.2 (CH_3), 20.7 (6×CH_3), 39.6 (2×CH_2), 52.2 (2×CH), 148.2 ($C_{C=O}$) ppm; (rotamer 2) 15.4 (CH_3), 20.2 (6×CH_3), 41.8 (2×CH_2), 51.6 (2×CH), 148.7 ($C_{C=O}$) ppm; **MS** (ES): *m/z* (%): 88 (100) $[C_5H_{14}N]^+$;

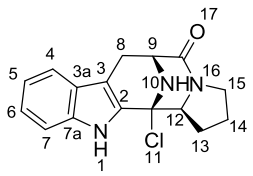
General procedure: Diketopiperazine **2** (1 equiv.) was suspended in dry CH_2Cl_2 and cooled with an ice-bath to 0 °C under a nitrogen atmosphere. Diphosgene (3 equiv.), dissolved in dry CH_2Cl_2, was added dropwise to the suspension. The mixture was heated to reflux. The reaction was monitored using HPLC-MS and after complete conversion, the organic phase was washed with saturated aq. $NaHCO_3$ and with water. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. Purification of the residue by chromatography provided the desired products **237**.

(6*S*,13*R*,13a*S*)-13-chloro-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-*b*]indol-5-one 237a.



Using the general procedure with isomer **2a** on a 0.7 mmol scale, compound **237a** was obtained after pTLC. **Yield** 40% (0.084 g); white powder; R_f=0.20 (CH_2Cl_2/4% MeOH); m.p. 224-230 °C; $[\alpha]_D^{25}$ = +102.8 (c=0.51 in $CHCl_3$); **^{1}H NMR (300 MHz, $CDCl_3$) δ** 1.76-2.16 (4H, m, C<u>H</u>$_2$-13, C<u>H</u>$_2$-14), 2.71 (1H, s, N<u>H</u>-10), 2.92-3.01 (1H, m, C<u>H</u>$_a$H$_b$-15), 3.05 (1H, d, *J*=16.4 Hz, C<u>H</u>$_a$H$_b$-8), 3.18 (1H, dd, *J*=16.4 Hz, *J*=5.9 Hz, CH$_a$<u>H</u>$_b$-8), 3.79 (1H, dd, *J*=11.0 Hz, *J*=5.0 Hz, C<u>H</u>-12), 4.04-4.15 (1H, m, CH$_a$<u>H</u>$_b$-15), 4.23 (1H, d, *J*=5.9 Hz, C<u>H</u>-9), 7.14 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C<u>H</u>-5), 7.24 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C<u>H</u>-6), 7.37 (1H, d, *J*=7.7 Hz, C<u>H</u>-7), 7.48 (1H, d, *J*=7.7 Hz, C<u>H</u>-4), 8.25 (1H, s, N<u>H</u>-1) ppm; **^{13}C NMR (75 MHz, $CDCl_3$) δ** 21.2 (CH_2-14), 24.9 (CH_2-8), 28.2 (CH_2-13), 44.4 (CH_2-15), 58.1 (CH-9), 68.5 (CH-12), 77.4 (C_q-11), 109.3 (C_q-3), 111.5 (CH-7), 119.3 (CH-4), 120.4 (CH-5), 123.6 (CH-6), 126.5 (C_q-3a), 134.3 (C_q-2), 136.0 (C_q-7a), 169.3 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3151 (NH), 1624 (C=O); **MS** (ES): *m/z* (%): 302/304 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{17}ClN_3O^+$ $[M + H]^+$: 302.1055; found: 302.1057.

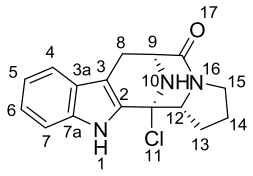
(6*R*,13*S*,13a*S*)-13-chloro-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 237b.



Apllying the general procedure on isomer **2b** on a 10 mmol scale, compound **237b** was obtained after column chromatography. **Yield** 50% (1.51 g); colourless crystals; R_f=0.25 (4/6 petroleum ether/EtOAc + 4% Et_3N); m.p. 248-250 °C; $[\alpha]_D^{25}$ = -115.3 (c=0.50 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.78-2.19 (4H, m, C$\underline{H_2}$-13, C$\underline{H_2}$-14), 2.70 (1H, s, N$\underline{H}$-10), 2.93-3.02 (1H, m, C$\underline{H_a}H_b$-15), 3.05 (1H, d, *J*=16.5 Hz, C$\underline{H_a}H_b$-8), 3.20 (1H, dd, *J*=16.5 Hz, *J*=6.6 Hz, C$H_a\underline{H_b}$-8), 3.80 (1H, dd, *J*=11.0 Hz, *J*=5.0 Hz, C$\underline{H}$-12), 4.05-4.16 (1H, m, C$H_a\underline{H_b}$-15), 4.24 (1H, d, *J*=6.6 Hz, C$\underline{H}$-9), 7.15 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.25 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.38 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.50 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 8.14 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.3 (CH_2-14), 24.9 (CH_2-8), 28.2 (CH_2-13), 44.4 (CH_2-15), 58.2 (CH-9), 68.5 (CH-12), 77.0 (C_q-11), 109.3 (C_q-3), 111.5 (CH-7), 119.3 (CH-4), 120.4 (CH-5), 123.6 (CH-6), 126.6 (C_q-3a), 134.3 (C_q-2), 136.0 (C_q-7a), 169.3 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3150 (NH), 1621 (C=O), 1461, 1450; **MS** (ES): *m/z* (%): 302/304 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{17}ClN_3O^+$ $[M + H]^+$: 302.1055; found: 302.1067.

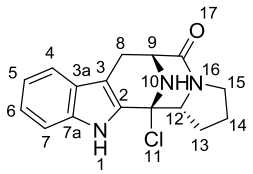
CCDC-1030976 and -1030977 contain the supplementary crystallographic data for this compound and can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* https://summary.ccdc.cam.ac.uk/structure-summary-form.

(6*S*,13*R*,13a*R*)-13-chloro-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 237c.



Using the general procedure on isomer **2c** on a 0.7 mmol scale, compound **237c** was obtained after pTLC. **Yield** 24% (0.051 g); white powder; R_f=0.17 (CH_2Cl_2/4% MeOH); m.p. 240-246 °C; $[\alpha]_D^{25}$ = +115.3 (c=0.50 in $CHCl_3$); **^{1}H NMR (300 MHz, $CDCl_3$) δ** 1.68-2.20 (4H, m, C$\underline{H_2}$-13, C$\underline{H_2}$-14), 2.69 (1H, s, N$\underline{H}$-10), 2.92-3.02 (1H, m, C$\underline{H_a}H_b$-15), 3.05 (1H, d, *J*=16.5 Hz, C$\underline{H_a}H_b$-8), 3.19 (1H, dd, *J*=16.5 Hz, *J*=6.3 Hz, C$H_a\underline{H_b}$-8), 3.79 (1H, dd, *J*=11.0 Hz, *J*=5.0 Hz, C$\underline{H}$-12), 4.03-4.16 (1H, m, C$H_a\underline{H_b}$-15), 4.24 (1H, d, *J*=6.3 Hz, C$\underline{H}$-9), 7.14 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.25 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-6), 7.37 (1H, d, *J*=7.5 Hz, C$\underline{H}$-7), 7.49 (1H, d, *J*=7.5 Hz, C$\underline{H}$-4), 8.20 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (75 MHz, $CDCl_3$) δ** 21.2 (CH_2-14), 24.9 (CH_2-8), 28.2 (CH_2-13), 44.4 (CH_2-15), 58.1 (CH-9), 68.5 (CH-12), 77.4 (C_q-11), 109.3 (C_q-3), 111.5 (CH-7), 119.3 (CH-4), 120.4 (CH-5), 123.6 (CH-6), 126.6 (C_q-3a), 134.3 (C_q-2), 136.0 (C_q-7a), 169.2 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3171 (NH), 1624 (C=O), 1451; MS (ES): *m/z* (%): 302/304 (100/35) $[M + H]^+$; HRMS (ESI): calcd. for $C_{16}H_{17}ClN_3O^+$ $[M + H]^+$: 302.1055; found: 302.1065.

(6*R*,13*S*,13a*R*)-13-chloro-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 237d.



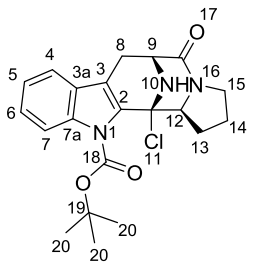
Following the general procedure on isomer **2d** on a 0.7 mmol scale, compound **237d** was obtained after pTLC. **Yield** 46% (0.097 g); white powder; R_f=0.22 (CH_2Cl_2/2% MeOH); m.p. 210-218 °C; $[\alpha]_D^{25}$ = -102.5 (c=0.51 in $CHCl_3$); **^{1}H NMR (300 MHz, $CDCl_3$) δ** 1.76-2.18 (4H, m, C$\underline{H_2}$-13, C$\underline{H_2}$-14), 2.68 (1H, s, N$\underline{H}$-10), 2.92-3.01 (1H, m, C$\underline{H_a}H_b$-15), 3.04 (1H, d, *J*=16.5 Hz, C$\underline{H_a}H_b$-8), 3.18 (1H, dd, *J*=16.5 Hz, *J*=6.3 Hz, $CH_a\underline{H_b}$-8), 3.79 (1H, dd, *J*=10.7 Hz, *J*=4.7 Hz, C$\underline{H}$-12), 4.03-4.15 (1H, m, $CH_a\underline{H_b}$-15), 4.23 (1H, d, *J*=6.3 Hz, C$\underline{H}$-9), 7.14 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-5), 7.24 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-6), 7.37 (1H, d, *J*=7.8 Hz, C$\underline{H}$-7), 7.48 (1H, d, *J*=7.8 Hz, C$\underline{H}$-4), 8.25 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (75 MHz, $CDCl_3$): δ** 21.3 (CH_2-14), 24.9 (CH_2-8), 28.2 (CH_2-13), 44.4 (CH_2-15), 58.2 (CH-9), 68.5 (CH-12), 77.0 (C_q-11), 109.3 (C_q-3), 111.5 (CH-7), 119.3 (CH-4), 120.4 (CH-5), 123.6 (CH-6), 126.6 (C_q-3a), 134.3 (C_q-2), 136.0 (C_q-7a), 169.3 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3222 (NH), 1613 (C=O), 1446; **MS** (ES): *m/z* (%): 302/304 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{17}ClN_3O^+$ $[M + H]^+$: 302.1055; found: 302.1062.

7.3. Introduction of protecting groups on the α-chloroamine

7.3.1. Boc protecting group

To a solution of α-chloroamine **237b** (1 equiv., 1.2 mmol, 0.35 g) in anhydrous ACN (5 mL) were added DMAP (0.02 equiv., 0.23 mmol, 28 mg) and $(Boc)_2O$ (3 equiv., 3.5 mmol, 0.76 g). After stirring for 3 hours at room temperature, the solvent was removed under reduced pressure and the residue was diluted with CH_2Cl_2 (15 mL). The organic phase was subsequently washed with brine (3×30 mL). Drying the organic phase over magnesium sulfate and concentration of the solvent under reduced pressure yielded the *N'*-protected α-chloroamine **241** as a yellow foam.

***tert*-Butyl (6*R*,13*S*,13a*S*)-13-chloro-5-oxo-1,2,3,5,6,7,13,13a-octahydro-12*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-12-carboxylate 241**



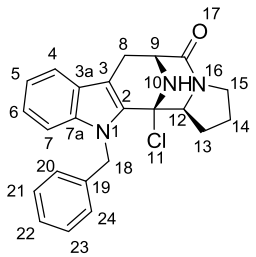
Yield 98% (0.46 g); yellow foam; m.p. 221-223 °C; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.71 (9H, s, 3×C$\underline{H_3}$-20), 1.85-2.08 (3H, m, C$\underline{H_a}H_b$-13, C$\underline{H_2}$-14), 2.18-2.28 (1H, m, C$\underline{H_a}H_b$-13), 2.49 (1H, s, N$\underline{H}$-10), 3.00-3.09 (1H, m, C$\underline{H_a}H_b$-15), 3.03 (1H, d, *J*=16.8 Hz, C$\underline{H_a}H_b$-8), 3.13 (1H, dd, *J*=16.8 Hz, *J*=6.3 Hz, $CH_a\underline{H_b}$-8), 4.09-4.19 (1H, m, $CH_a\underline{H_b}$-15), 4.19 (1H, d, *J*=6.3 Hz, C$\underline{H}$-9), 4.61 (1H, dd, *J*=10.9 Hz, *J*=4.9 Hz, C$\underline{H}$-12), 7.25 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-6), 7.35 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-5), 7.45 (1H, d, *J*=7.6 Hz, C$\underline{H}$-7), 7.94 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.4 (CH_2-14), 25.3 (CH_2-8), 27.5 (CH_2-13), 28.2 (3×CH_3-20), 44.3 (CH_2-15), 56.1 (CH-9), 66.4 (CH-12),

76.1 (C_q-11), 84.7 (C_q-19), 114.8 (CH-4), 116.4 (C_q-3), 119.0 (CH-7), 122.9 (CH-6), 125.7 (CH-5), 127.1 (C_q-3a), 135.3 (C_q-2), 136.1 (C_q-7a), 149.8 ($C_{C=O}$-18), 168.7 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 1735 (C=O), 1638 (C=O), 1452; **MS** (ES): *m/z* (%): 402/404 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{21}H_{25}ClN_3O_3^+$ $[M + H]^+$: 402.1579; found: 402.1590.

7.3.2. Benzyl protecting group

A solution of α-chloroamine **237b** (1 equiv., 0.33 mmol, 100 mg) in dry DMF (5 mL) was cooled to 0 °C and sodium hydride (2.5 equiv., 0.83 mmol, 33 mg) was added. After 15 minutes benzyl bromide (2.5 equiv., 0.83 mmol, 0.10 mL) was added to the solution. The mixture was allowed to warm to room temperature and was stirred for one day. Then, EtOAc (15 mL) was added and the organic phase was washed with brine (5×10 mL) and water (2×10 mL). Drying the organic phase over magnesium sulfate and concentration of the solvent under reduced pressure yielded the crude product. Compound **244** was isolated with pTLC as a white foam.

(6*R*,13*S*,13a*S*)-12-benzyl-13-chloro-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 244



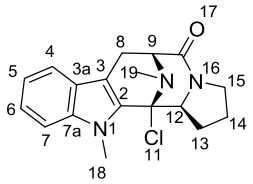
Yield 56% (73 mg); white foam; m.p. 209-211 °C; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.50-1.62 (1H, m, C$\underline{H}_a$$H_b$-14), 1.80-1.95 (3H, m, C$\underline{H}_2$-13, CH$_a$$\underline{H}_b$-14), 2.67 (1H, s, N$\underline{H}$-10), 2.27-2.80 (1H, m, C$\underline{H}_a$$H_b$-15), 3.13 (1H, d, *J*=16.3 Hz, C$\underline{H}_a$$H_b$-8), 3.26 (1H, dd, *J*=16.3 Hz, *J*=6.5 Hz, CH$_a$$\underline{H}_b$-8), 3.38 (1H, dd, *J*=11.2 Hz, *J*=5.2 Hz, C$\underline{H}$-12), 3.96-4.20 (1H, m, CH$_a$$\underline{H}_b$-15), 4.19 (1H, d, *J*=6.5 Hz, C$\underline{H}$-9), 5.66 (1H, d, *J*=17.0 Hz, C$\underline{H}_a$$H_b$-18), 5.74 (1H, d, *J*=17.0 Hz, CH$_a$$\underline{H}_b$-18), 6.98-7.02 (2H, m, C$\underline{H}$-20, C$\underline{H}$-24), 7.12-7.27 (6H, m, C$\underline{H}$-5, C$\underline{H}$-6, C$\underline{H}$-7, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23), 7.54 (1H, d, *J*=7.9 Hz, C$\underline{H}$-4) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.2 (CH_2-14), 25.7 (CH_2-8), 27.8 (CH_2-13), 44.1 (CH_2-15), 47.9 (CH_2-18), 57.7 (CH-9), 67.5 (CH-12), 77.0 (C_q-11), 110.2 (CH-7), 110.4 (C_q-3), 119.3 (CH-4), 120.3 (CH-5), 123.7 (CH-6), 125.6 (C_q-3a), 126.3 (CH-20, CH-24), 127.4 (CH-22), 128.7 (CH-21, CH-23), 133.9 (C_q-2), 137.6 (C_q-7a*), 137.8 (C_q-19*), 169.0 ($C_{C=O}$-17) ppm (Signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 1632 (C=O), 1454; **MS** (ES): *m/z* (%): 392/394 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{23}H_{23}ClN_3O^+$ $[M + H]^+$: 392.1524; found: 392.1532.

7.3.3. Methyl groups

A solution of α-chloroamine **237b** (1 equiv., 2.0 mmol, 604 mg) in dry DMF (10 mL) was cooled to 0 °C and sodium hydride (2.5 equiv., 5.0 mmol, 200 mg) was added. After 15 minutes methyl iodide (2.5 equiv., 5.0 mmol, 0.31 mL)was added. The mixture was allowed to warm to room temperature and was stirred for one day. Then EtOAc (20 mL) was added and the organic phase was washed with

brine (5×15 mL) and water (2×15 mL). Drying the organic phase over magnesium sulfate and removal of the solvent under reduced pressure yielded the crude product. Compound **247** was isolated as a white foam *via* column chromatography using EtOAc as eluent.

(6*R*,13*S*,13a*S*)-13-chloro-12,14-dimethyl-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 247



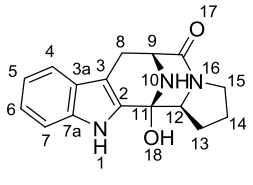
Yield 48% (317 mg); white foam; m.p. 230-232 °C; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.75-1.90 (1H, m, C$\underline{H}_a$H$_b$-14), 1.95-2.22 (3H, m, C$\underline{H}_2$-13, CH$_a\underline{H}_b$-14), 2.68 (3H, s, C$\underline{H}_3$-19), 2.92 (1H, d, *J*=16.8 Hz, C$\underline{H}_a$H$_b$-8), 2.89-2.98 (1H, m, C$\underline{H}_a$H$_b$-15), 3.35 (1H, dd, *J*=16.8 Hz, *J*=6.4 Hz, CH$_a\underline{H}_b$-8), 3.74 (1H, dd, *J*=11.1 Hz, *J*=5.1 Hz, C$\underline{H}$-12), 3.94 (3H, s, C$\underline{H}_3$-18), 4.03 (1H, d, *J*=6.4 Hz, C$\underline{H}$-9), 4.09-4.18 (1H, m, CH$_a\underline{H}_b$-15), 7.13 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.28 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.34 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.51 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 19.8 (CH_2-8), 21.5 (CH_2-14), 28.6 (CH_2-13), 32.3 (CH_3-18), 36.6 (CH_3-19), 44.4 (CH_2-15), 64.2 (CH-9), 68.8 (CH-12), 82.2 (C_q-11), 108.6 (C_q-3), 109.4 (CH-7), 119.2 (CH-4), 119.9 (CH-5), 123.2 (CH-6), 125.3 (C_q-3a), 131.3 (C_q-2), 137.9 (C_q-7a), 169.4 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 1634 (C=O), 1466; **MS** (ES): *m/z* (%): 330/332 (100/33) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{18}H_{21}ClN_3O^+$ $[M + H]^+$: 330.1368; found: 330.1372.

7.4. Synthesis of α-chloroamine derivatives

7.4.1. Procedure for the synthesis of derivative 253

α-Chloroamine **237b** (1.0 equiv., 1 mmol, 302 mg) was suspended in 10 mL of 2 M aq. NaOH and refluxed until the conversion was complete. The aqueous phase was then neutralized with 2 M HCl (10 mL), resulting in a suspension which was extracted three times with chloroform (10 mL). The white precipitate moved to the organic phase (suspension). The layers were separated and the organic phase was filtered off to yield compound **253** as a white powder (65%).

(6*R*,13*S*,13a*S*)-13-hydroxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 253



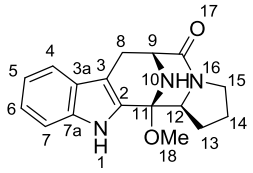
Yield 65% (184 mg); white powder; m.p. >260 °C; $[\alpha]_D^{20}$ = +92.8 (c=0.42 in DMF); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.21-1.34 (1H, m, C$\underline{H}_a$H$_b$-13), 1.54-1.72 (2H, m, C$\underline{H}_2$-14), 2.04-2.13 (1H, m, CH$_a\underline{H}_b$-13), 2.81 (1H, dd, *J*=15.1 Hz, *J*=1.4 Hz, C$\underline{H}_a$H$_b$-8), 2.91 (1H, dd, *J*=15.1 Hz, *J*=5.1 Hz, CH$_a\underline{H}_b$-8), 3.07 (1H, ddd, *J*=11.6 Hz, *J*=9.3 Hz, *J*=9.3 Hz, C$\underline{H}_a$H$_b$-15), 3.17 (1H, ddd, *J*=11.6 Hz, *J*=9.0 Hz, *J*=2.5 Hz, CH$_a\underline{H}_b$-15), 3.34 (1H, s, N$\underline{H}$-10), 3.79 (1H, dd, *J*=11.2 Hz, *J*=5.1 Hz, C$\underline{H}$-12), 3.85 (1H, d, *J*=5.1 Hz, C$\underline{H}$-9), 6.48 (1H, s, O$\underline{H}$-18),

6.93 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.0 Hz, CH-5), 7.04 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.0 Hz, CH-6), 7.31 (1H, d, *J*=7.7 Hz, CH-7), 7.37 (1H, d, *J*=7.7 Hz, CH-4), 11.04 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 21.7 (CH_2-14), 26.6 (CH_2-8), 28.2 (CH_2-13), 44.7 (CH_2-15), 56.1 (CH-9), 68.2 (CH-12), 80.1 (C_q-11), 108.2 (C_q-3), 111.5 (CH-7), 118.1 (CH-4), 118.3 (CH-5), 121.0 (CH-6), 126.1 (C_q-3a), 133.6 (C_q-2), 136.0 (C_q-7a), 169.9 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3262 (NH), 1593 (C=O), 1452; **MS** (ES): *m/z* (%) = 284 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{18}N_3O_2^+$ $[M + H]^+$: 284.1394; found: 284.1401.

7.4.2. Procedure for the synthesis of derivative 240

α-Chloroamine **237b** (1 equiv., 1 mmol, 302 mg) was suspended in MeOH (10 mL). A sodium methoxide solution in MeOH (1 equiv., 1 mmol, 0.25 mL of 4 M solution) was added and the mixture was refluxed for two and a half hours. Next, the reaction was quenched by the addition of water and the solvent was evaporated under reduced pressure. The residual white precipitate was redissolved in chloroform (10 mL) and washed three times with water. After drying the organic phase over $MgSO_4$, the solvent was removed by evaporation to give compound **240** as a white solid (96%).

(6*R*,13*S*,13a*S*)-13-methoxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 240

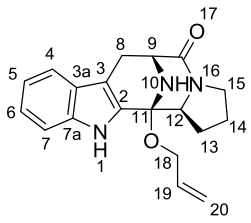


Yield 96% (285 mg); white solid; m.p. >260 °C; $[\alpha]_D^{20}$ = -69.8 (c=0.35 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.55-1.68 (1H, m, CH_aH_b-13), 1.72-1.86 (1H, m, CH_aH_b-14), 1.92-2.04 (2H, m, CH_aH_b-13, CH_aH_b-14), 2.23 (1H, s, NH-10), 2.93 (1H, ddd, *J*=12.2 Hz, *J*=10.1 Hz, *J*=4.8 Hz, CH_aH_b-15), 3.02 (1H, dd, *J*=15.9 Hz, *J*=1.3 Hz, CH_aH_b-8), 3.20 (1H, dd, *J*=15.9 Hz, *J*=6.5 Hz, CH_aH_b-8), 3.35 (3H, s, CH_3-18), 3.58 (1H, dd, *J*=11.8 Hz, *J*=4.9 Hz, CH-12), 3.94-4.03 (1H, m, CH_aH_b-15); 4.17 (1H, dd, *J*=6.5 Hz, *J*=1.3 Hz, CH-9), 7.13 (1H, ddd, *J*=7.8 Hz, *J*=7.8 Hz, *J*=1.0 Hz, CH-5), 7.21 (1H, ddd, *J*=7.8 Hz, *J*=7.8 Hz, *J*=1.0 Hz, CH-6), 7.36 (1H, d, *J*=7.8 Hz, CH-7), 7.50 (1H, d, *J*=7.8 Hz, CH-4), 8.09 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.5 (CH_2-14), 24.4 (CH_2-8), 25.5 (CH_2-13), 43.5 (CH_2-15), 51.3 (CH_3-18), 57.8 (CH-9), 66.6 (CH-12), 83.6 (C_q-11), 111.2 (C_q-3), 111.3 (CH-7), 119.0 (CH-4), 120.0 (CH-5), 122.8 (CH-6), 126.9 (C_q-3a), 133.2 (C_q-2), 136.1 (C_q-7a), 171.3 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3150 (NH), 1620 (C=O), 1462; **MS** (ES): *m/z* (%) = 298 (100) $[M + H]^+$, 595 (75); **HRMS** (ESI): calcd. for $C_{17}H_{20}N_3O_2^+$ $[M + H]^+$: 298.1550; found: 298.1556.

7.4.3. General procedure for the synthesis of derivatives 254-262 and 265

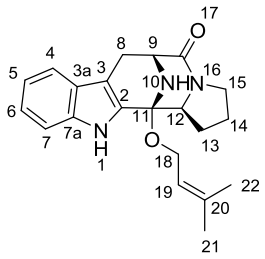
The nucleophile (3 equiv., 1.5 mmol or 1.5 equiv., 0.75 mmol) was dissolved in THF (10 mL) at room temperature. The solution was cooled to 0 °C and sodium hydride (3 equiv., 1.5 mmol, 60 mg or 1.5 equiv., 0.75 mmol, 30 mg respectively, 60% in mineral oil) was added. After stirring for 15 minutes at 0 °C, α-chloroamine **237b** (1 equiv., 0.5 mmol, 151 mg) was added to the mixture. The mixture was allowed to warm up to room temperature and was stirred until the conversion was complete. An ammonia chloride solution (10 mL) and EtOAc (15 mL) were added subsequently. The layers were separated and the aqueous phase was extracted with 10 mL EtOAc. The combined organic phases were washed 3 times with water (10 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. When necessary, further purification was done by normal-phase chromatography using (a mixture of) EtOAc (and methanol) as eluent or reversed-phase chromatography using a gradient of water and acetonitrile as eluent to provide the desired compound.

(6*R*,13*S*,13a*S*)-13-allyloxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 254



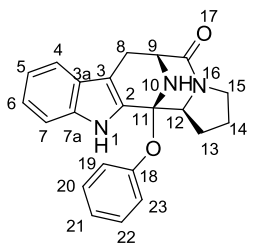
Using the general procedure with 3 equivalents of allyl alcohol, compound **254** was obtained without further purification. **Yield** 86% (140 mg); yellow powder; m.p. 258-260 °C; $[\alpha]_D^{20}$ = -28.4 (c=0.32 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.58-1.71 (1H, m, CH_aH_b-13), 1.71-1.83 (1H, m, CH_aH_b-14), 1.91-2.05 (2H, m, CH_aH_b-13, CH_aH_b-14), 2.60 (1H, s, NH-10), 2.90 (1H, ddd, *J*=12.1 Hz, *J*=10.3 Hz, *J*=4.6 Hz, CH_aH_b-15), 3.01 (1H, dd, *J*=15.9 Hz, *J*=1.0 Hz, CH_aH_b-8), 3.18 (1H, dd, *J*=15.9 Hz, *J*=6.4 Hz, CH_aH_b-8), 3.61 (1H, dd, *J*=11.7 Hz, *J*=4.9 Hz, CH-12), 3.92 (1H, ddt, *J*=13.7 Hz, *J*=5.1 Hz, *J*=1.7 Hz, CH_aH_b-18), 3.96 (1H, ddd, *J*=12.1 Hz, *J*=9.4 Hz, *J*=6.1 Hz, CH_aH_b-15), 4.15 (1H, dd, *J*=5.1 Hz, *J*=1.0 Hz, CH-9), 4.31 (ddt, *J*=13.7 Hz, *J*=4.9 Hz, *J*=1.7 Hz, 1H, CH_aH_b-18), 5.14 (1H, ddt, *J*=10.5 Hz, *J*=1.7 Hz, *J*=1.7 Hz, CH_aH_b-20), 5.28 (1H, ddt, *J*=17.1 Hz, *J*=1.7 Hz, *J*=1.7 Hz, CH_aH_b-20), 5.92 (1H, dddd, *J*=17.1 Hz, *J*=10.5 Hz, *J*=5.1 Hz, *J*=4.9 Hz, CH-19), 7.10 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=0.6 Hz, CH-5), 7.18 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=0.6 Hz, CH-6), 7.33 (1H, d, *J*=7.7 Hz, CH-7), 7.48 (1H, d, *J*=7.7 Hz, CH-4), 8.45 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.5 (CH_2-14), 24.4 (CH_2-8), 25.6 (CH_2-13), 43.6 (CH_2-15), 57.9 (CH-9), 64.4 (CH_2-18), 66.7 (CH-12), 83.6 (C_q-11), 110.8 (C_q-3), 111.4 (CH-7), 115.6 (CH_2-20), 119.0 (CH-4), 119.9 (CH-5), 122.7 (CH-6), 126.9 (C_q-3a), 133.6 (C_q-2), 135.3 (CH-19), 136.2 (C_q-7a), 171.4 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3266 (NH), 1628 (C=O), 1457; **MS** (ES): *m/z* (%) = 324 (100) $[M + H]^+$, 647 (65); **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3O_2^+$ $[M + H]^+$: 324.1707; found: 324.1700.

(6*R*,13*S*,13a*S*)-13-(3-methylbut-2-en-1-yl)oxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino[4,5-b]indol-5-one 255



Following the general procedure using 1.5 equivalents of prenyl alcohol, **255** was obtained after purification by reversed-phase chromatography (2 column volumes (CVs) 10% ACN, over 16 CVs to 40% ACN then during 8 CVs 40% ACN) as a yellow powder. **Yield** 33% (58 mg); yellow powder; m.p. 116-124 °C; $[\alpha]_D^{20}$ = -66.5 (c=0.53 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.53 (3H, s, C$\underline{H}_3$-21*), 1.57-1.70 (1H, m, C$\underline{H}_a$H$_b$-13), 1.73 (3H, s, C$\underline{H}_3$-22*), 1.71-1.84 (1H, m, CH$_a$H$_b$-14), 1.93-2.04 (2H, m, CH$_a\underline{H}_b$-13, CH$_a\underline{H}_b$-14), 2.31 (1H, s, N$\underline{H}$-10), 2.88-2.96 (1H, m, CH$_a$H$_b$-15), 3.02 (1H, d, *J*=15.9 Hz, C$\underline{H}_a$H$_b$-8), 3.20 (1H, dd, *J*=15.9 Hz, *J*=6.0 Hz, CH$_a\underline{H}_b$-8), 3.58 (1H, dd, *J*=11.8 Hz, *J*=4.9 Hz, C$\underline{H}$-12), 3.90-4.02 (2H, m, C$\underline{H}_a$H$_b$-18, CH$_a\underline{H}_b$-15), 4.17 (1H, d, *J*=6.0 Hz, C$\underline{H}$-9), 4.27 (1H, dd, *J*=11.9 Hz, *J*=6.5 Hz, CH$_a\underline{H}_b$-18), 5.34 (1H, t, *J*=6.5 Hz, C$\underline{H}$-19), 7.12 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.21 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.35 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.35 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 8.10 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 18.1 (CH_3-21*), 21.5 (CH_2-14), 24.5 (CH_2-8), 25.6 (CH_2-13), 25.8 (CH_3-22*), 43.6 (CH_2-15), 57.9 (CH-9), 60.7 (CH_2-18), 66.7 (CH-12), 83.4 (C_q-11), 110.9 (C_q-3), 111.2 (CH-7), 119.0 (CH-4), 119.9 (CH-5), 121.3 (CH-19), 122.7 (CH-6), 126.9 (C_q-3a), 133.8 (C_q-2), 136.1 (C_q-7a**), 136.5 (C_q-20**), 171.3 ($C_{C=O}$-17) ppm (The signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 3252 (NH), 1627 (C=O), 1446; **MS** (ES): *m/z* (%) = 352 (100) $[M + H]^+$, 703 (30); **HRMS** (ESI): calcd. for $C_{21}H_{26}N_3O_2^+$ $[M + H]^+$: 352.2020; found: 352.2029.

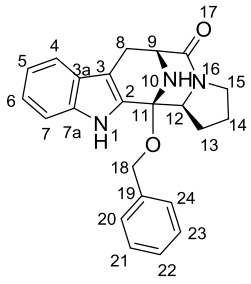
(6*R*,13*S*,13a*S*)-13-phenoxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 256



Following the general procedure using 3 equivalents of phenol, **256** was obtained as the insoluble residue from rinsing the crude with acetone and methanol. **Yield** 19% (55 mg); white powder; m.p. 136-140 °C; $[\alpha]_D^{20}$ = -34.1 (c=0.26 in THF); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.74-1.87 (1H, m, C$\underline{H}_a$H$_b$-14), 1.88-2.10 (3H, m, C$\underline{H}_2$-13, CH$_a\underline{H}_b$-14), 2.79 (1H, d, *J*=15.7 Hz, C$\underline{H}_a$H$_b$-8), 2.87 (1H, ddd, *J*=11.7 Hz, *J*=10.2 Hz, *J*=4.8 Hz, C$\underline{H}_a$H$_b$-15), 3.08 (1H, dd, *J*=15.7 Hz, *J*=6.1 Hz, CH$_a\underline{H}_b$-8), 3.71 (1H, dd, *J*=11.2 Hz, *J*=5.3 Hz, C$\underline{H}$-12), 3.82 (1H, ddd, *J*=11.7 Hz, *J*=9.2 Hz, *J*=6.2 Hz, CH$_a\underline{H}_b$-15), 3.95 (1H, ddd, *J*=6.1 Hz, *J*=4.1 Hz, *J*=1.3 Hz, C$\underline{H}$-9), 4.10 (1H, d, *J*=4.1 Hz, N$\underline{H}$-10), 6.86 (1H, td, *J*=6.8 Hz, *J*=1.7 Hz, C$\underline{H}$-21), 6.98 (1H, ddd, *J*=7.8 Hz, *J*=7.8 Hz, *J*=1.0 Hz, C$\underline{H}$-5), 7.04-7.14 (5H,m, 1H, m, C$\underline{H}$-6, C$\underline{H}$-19, C$\underline{H}$-20, C$\underline{H}$-22, C$\underline{H}$-23), 7.26 (1H, d, *J*=7.8 Hz, C$\underline{H}$-7), 7.43 (1H, d, *J*=7.8 Hz, C$\underline{H}$-4), 11.14 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 21.2 (CH_2-14), 23.9 (CH_2-8), 24.9 (CH_2-13), 43.3 (CH_2-15), 57.5 (CH-9), 66.9 (CH-12), 85.1 (C_q-11), 108.3 (C_q-3), 111.7 (CH-7), 118.3 (CH-4), 118.8 (CH-5), 119.4 (CH-

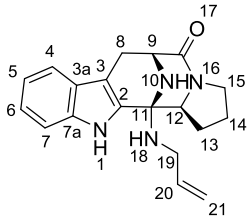
19, CH-23), 121.6 (CH-6, CH-21), 126.1 (C_q-3a), 128.6 (CH-20, CH-22), 134.3 (C_q-2), 136.3 (C_q-7a), 155.0 (C_q-18), 170.6 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3164 (NH), 1610 (C=O), 1456; **MS** (ES): *m/z* (%) = 360 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{22}H_{22}N_3O_2^+$ $[M + H]^+$: 360.1707; found: 360.1700.

(6*R*,13*S*,13a*S*)-13-benzyloxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 257



Following the general procedure using 1.5 equivalents of benzyl alcohol, **257** was obtained *via* purification by pTLC as a white solid. **Yield** 26% (49 mg); white solid; R_f=0.41 (EtOAc); m.p. 134-138 °C; $[\alpha]_D^{20}$ = -20.9 (c=0.42 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.67-1.86 (2H, m, C$\underline{H}_a$$H_b$-13, C$\underline{H}_a$$H_b$-14), 1.96-2.12 (2H, m, C$H_a\underline{H}_b$-13, C$H_a\underline{H}_b$-14), 2.37 (1H, s, N$\underline{H}$-10), 2.95 (1H, ddd, *J*=12.2 Hz, *J*=10.2 Hz, *J*=4.7 Hz, C$\underline{H}_a$$H_b$-15), 3.04 (1H, dd, *J*=15.9 Hz, *J*=1.4 Hz, C$\underline{H}_a$$H_b$-8), 3.23 (1H, dd, *J*=15.9 Hz, *J*=6.5 Hz, C$H_a\underline{H}_b$-8), 3.66 (1H, dd, *J*=11.7 Hz, *J*=4.9 Hz, C$\underline{H}$-12), 3.99 (1H, ddd, *J*=12.2 Hz, *J*=9.4 Hz, *J*=6.1 Hz, C$H_a\underline{H}_b$-15), 4.18 (1H, dd, *J*=6.1 Hz, *J*=1.4 Hz, C$\underline{H}$-9), 4.45 (1H, d, *J*=12.5 Hz, C$\underline{H}_a$$H_b$-18), 4.91 (1H, d, *J*=12.5 Hz, C$H_a\underline{H}_b$-18), 7.14 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.1 Hz, C$\underline{H}$-5), 7.21 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.1 Hz, C$\underline{H}$-6), 7.29-7.38 (6H, m, C$\underline{H}$-7, C$\underline{H}$-20, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24), 7.52 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4), 8.05 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.5 (CH_2-14), 24.4 (CH_2-8), 25.7 (CH_2-13), 43.6 (CH_2-15), 57.9 (CH-9), 65.4 (CH_2-18), 66.7 (CH-12), 83.8 (C_q-11), 111.2 (C_q-3), 111.4 (CH-7), 119.0 (CH-4), 120.0 (CH-5), 122.8 (CH-6), 126.9 (CH-20, CH-24, C_q-3a), 127.5 (CH-22), 128.5 (CH-21, CH-23), 133.4 (C_q-2), 136.1 (C_q-7a), 138.7 (C_q-19), 171.3 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3258 (NH), 1620 (C=O), 1454; **MS** (ES): *m/z* (%) = 374 (100) $[M + H]^+$, 747 (25); **HRMS** (ESI): calcd. for $C_{23}H_{24}N_3O_2^+$ [M + H]+: 374.1863; found: 374.1861.

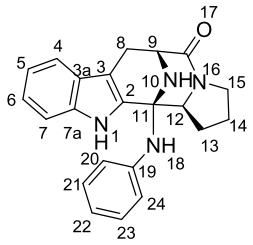
(6*R*,13*R*,13a*S*)-13-allylamino-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 258



Using the general procedure using 3 equivalents of allylamine, compound **258** was obtained without further purification. **Yield** 77% (124 mg); yellow powder; m.p. 206-210 °C; $[\alpha]_D^{20}$ = -28.3 (c=0.34 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.50-1.73 (2H, m, C$\underline{H}_a$$H_b$-13, N$\underline{H}$-10), 1.73-1.88 (1H, m, C$\underline{H}_a$$H_b$-14), 1.88-1.97 (1H, m, C$H_a\underline{H}_b$-13), 1.97-2.12 (2H, m, 1H, m, C$H_a\underline{H}_b$-14, N$\underline{H}$-18), 2.94 (1H, ddd, *J*=12.2 Hz, *J*=10.2 Hz, *J*=4.6 Hz, C$\underline{H}_a$$H_b$-15), 3.03 (1H, dd, *J*=16.1 Hz, *J*=1.4 Hz, C$\underline{H}_a$$H_b$-8), 3.02-3.13 (1H, m, C$\underline{H}_a$$H_b$-19), 3.13 (1H, dd, *J*=16.1 Hz, *J*=6.4 Hz, C$H_a\underline{H}_b$-8), 3.39 (1H, ddt, *J*=14.6 Hz, *J*=6.4 Hz, *J*=1.5 Hz, C$H_a\underline{H}_b$-19), 3.50 (1H, dd, *J*=11.9 Hz, *J*=4.6 Hz, C$\underline{H}$-12), 4.01 (1H, ddd, *J*=12.2 Hz, *J*=9.5 Hz, *J*=6.2 Hz, C$H_a\underline{H}_b$-15), 4.18 (1H, dd, *J*=6.4 Hz, *J*=1.4 Hz, C$\underline{H}$-9), 5.11 (1H, ddt, *J*=10.3 Hz, *J*=1.5 Hz,

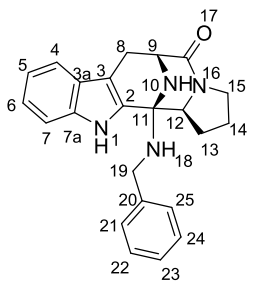
J=1.5 Hz, C$\underline{H}_a$H$_b$-21), 5.23 (1H, ddt, *J*=17.1 Hz, *J*=1.5 Hz, *J*=1.5 Hz, CH$_a\underline{H}_b$-21), 5.92 (1H, dddd, *J*=17.1 Hz, *J*=10.3 Hz, *J*=6.4 Hz, *J*=4.8 Hz, C$\underline{H}$-20), 7.11 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.0 Hz, C$\underline{H}$-5), 7.19 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.0 Hz, C$\underline{H}$-6), 7.35 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.49 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 8.20 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, CDCl$_3$) δ** 21.5 (CH_2-14), 25.1 (CH_2-8, CH_2-13), 43.2 (CH_2-15), 44.7 (CH_2-19), 57.1 (CH-9), 67.1 (CH-12), 67.8 (C_q-11), 110.0 (C_q-3), 111.2 (CH-7), 115.6 (CH_2-21), 118.8 (CH-4), 119.7 (CH-5), 122.5 (CH-6), 127.3 (C_q-3a), 134.9 (C_q-2), 135.6 (C_q-7a), 136.9 (CH-20), 170.9 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3273 (NH), 1613 (C=O), 1455; **MS** (ES): *m/z* (%) = 323 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{19}H_{23}N_4O^+$ $[M + H]^+$: 323.1866; found: 323.1881.

(6*R*,13*S*,13a*S*)-13-phenylamino-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 259



Following the general procedure using 3 equivalents of aniline, **259** was obtained as the insoluble residue from rinsing the crude with acetonitrile. **Yield** 24% (43 mg); brown powder; m.p. 198-202 °C; $[\alpha]_D^{20}$ = +108.9 (c=0.21 in THF); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.78-1.82 (1H, m, C$\underline{H}_a$H$_b$-14), 1.82-1.98 (2H, m, 1H, m, C$\underline{H}_a$H$_b$-13, CH$_a\underline{H}_b$-14), 2.12-2.21 (1H, m, CH$_a\underline{H}_b$-13), 2.74-2.86 (2H, m, C$\underline{H}_a$H$_b$-8, C$\underline{H}_a$H$_b$-15), 3.07 (1H, dd, *J*=15.5 Hz, *J*=6.2 Hz, CH$_a\underline{H}_b$-8), 3.51 (1H, d, *J*=3.8 Hz, N$\underline{H}$-10), 3.53 (1H, dd, *J*=11.2 Hz, *J*=4.7 Hz, C$\underline{H}$-12), 3.78-3.88 (2H, m, C$\underline{H}$-9, CH$_a\underline{H}_b$-15), 5.51 (1H, s, N$\underline{H}$-18), 6.44-6.56 (1H, m, C$\underline{H}$-22), 6.86-6.92 (4H, m, C$\underline{H}$-20, C$\underline{H}$-21, C$\underline{H}$-23, C$\underline{H}$-24), 6.94 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.2 Hz, C$\underline{H}$-5), 7.00 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.2 Hz, C$\underline{H}$-6), 7.25 (1H, d, *J*=7.6 Hz, C$\underline{H}$-7), 7.39 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4), 10.63 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 21.2 (CH_2-14), 24.4 (CH_2-8), 24.6 (CH_2-13), 42.8 (CH_2-15), 56.0 (CH-9), 66.1 (C_q-11), 67.2 (CH-12), 107.4 (C_q-3), 111.6 (CH-7), 116.2 (CH-20, CH-24), 117.3 (CH-22), 117.9 (CH-4), 118.5 (CH-5), 120.9 (CH-6), 126.5 (C_q-3a), 127.9 (CH-21, CH-23), 135.8 (C_q-7a), 136.9 (C_q-2), 145.8 (C_q-19), 170.9 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3195 (NH), 1601 (C=O), 1498, 1456; **MS** (ES): *m/z* (%) = 359 (100) $[M + H]^+$, 717 (25); **HRMS** (ESI): calcd. for $C_{22}H_{23}N_4O^+$ $[M + H]^+$: 359.1866; found: 359.1877.

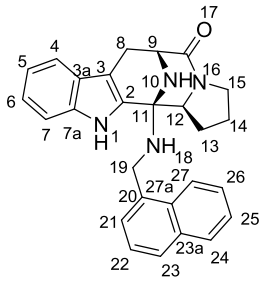
(6*R*,13*R*,13a*S*)-13-benzylamino-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 260



Following the general procedure using 1.5 equivalents of benzylamine, **260** was obtained on purification by pTLC as a white powder. **Yield** 28% (52 mg); white powder ; R_f=0.37 (EtOAc + 5% MeOH); m.p. 142-148 °C; $[\alpha]_D^{20}$ = +0.4 (c=0.40 in $CHCl_3$); **^{1}H NMR (400 MHz, CDCl$_3$) δ** 1.58-1.72 (1H, m, C$\underline{H}_a$H$_b$-13), 1.72-1.85 (1H, m, C$\underline{H}_a$H$_b$-14), 1.85-2.21 (4H, m, N$\underline{H}$-10, CH$_a\underline{H}_b$-13, CH$_a\underline{H}_b$-14,

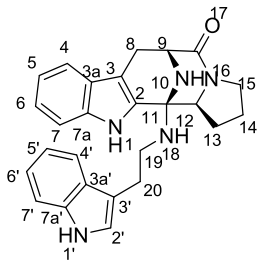
NH-18), 2.92 (1H, ddd, *J*=12.2 Hz, *J*=10.2 Hz, *J*=4.6 Hz, CH_aH_b-15), 3.05 (1H, dd, *J*=16.1 Hz, *J*=1.3 Hz, CH_aH_b-8), 3.17 (1H, dd, *J*=16.1 Hz, *J*=6.5 Hz, CH_aH_b-8), 3.53 (1H, dd, *J*=11.8 Hz, *J*=4.6 Hz, CH-12), 3.62 (1H, d, *J*=13.5 Hz, CH_aH_b-19), 3.98 (1H, d, *J*=13.5 Hz, CH_aH_b-19), 3.95-4.03 (1H, m, CH_aH_b-15), 4.19 (1H, dd, *J*=6.4 Hz, *J*=1.3 Hz, CH-9), 7.11 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.1 Hz, CH-5), 7.19 (1H, ddd, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.1 Hz, CH-6), 7.25-7.36 (6H, m, CH-7, CH-21, CH-22, CH-23, CH-24, CH-25), 7.50 (1H, d, *J*=7.6 Hz, CH-4), 8.32 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.5 (CH_2-14), 25.1 (CH_2-8*), 25.2 (CH_2-13*), 43.2 (CH_2-15), 46.2 (CH_2-19), 57.1 (CH-9), 67.2 (CH-12), 67.9 (C_q-11), 110.1 (C_q-3), 111.2 (CH-7), 118.7 (CH-4), 119.7 (CH-5), 122.5 (CH-6), 127.2 (CH-23), 127.3 (C_q-3a), 127.7 (CH-21, CH-25), 128.6 (CH-22, CH-24), 134.8 (C_q-2), 135.7 (C_q-7a), 140.2 (C_q-20), 170.9 ($C_{C=O}$-17) ppm (The signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 3291 (NH), 1622 (C=O), 1456; **MS** (ES): *m/z* (%) = 373 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{23}H_{25}N_4O^+$ [M + H]+: 373.2023; found: 373.2019.

(6*R*,13*R*,13a*S*)-13-((naphthalen-1-ylmethyl)amino)-1,2,3,6,7,12,13,13a-octahydro-5H-6,13-epiminopyrrolo[1',2':1,2]azocino[4,5-b]indol-5-one 261



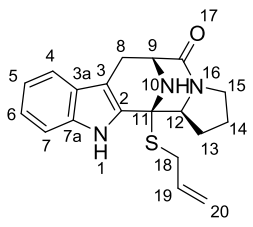
Following the general procedure using 3 equivalents of 1-napthylmethylamine, **261** was obtained on purification by reversed-phase chromatography using a H_2O/ACN gradient (during 10 CV 30% ACN, over 20 CV to 80% ACN) as a white powder. **Yield** 36% (76 mg); white powder ; m.p. >260 °C; $[\alpha]_D^{25}$ = -44.5 (c=0.64 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.60-2.06 (5H, m, CH_2-13, CH_2-14, NH-10*), 2.22 (1H, br s, NH-18*), 2.90-2.98 (1H, m, CH_aH_b-15), 3.09 (1H, d, *J*=15.9 Hz, CH_aH_b-8), 3.24 (1H, dd, *J*=15.9 Hz, *J*=6.3 Hz, CH_aH_b-8), 3.52-3.59 (H, m, CH-12), 3.96-4.06 (2H, m, CH_aH_b-15, CH_aH_b-19), 4.24 (1H, d, *J*=6.3 Hz, CH-9), 4.53 (1H, d, *J*=13.9 Hz, CH_aH_b-19), 7.13 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, CH-5), 7.21 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, CH-6), 7.33 (1H, d, *J*=7.6 Hz, CH-7), 7.42-7.57 (5H, m, CH-4, CH-21, CH-22, CH-25, CH-26), 7.79 (1H, d, *J*=8.2 Hz, CH-23), 7.85-7.90 (1H, m, CH-24), 7.94-8.00 (1H, m, CH-27), 8.19 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.5 (CH_2-14), 25.1 (CH_2-8, CH_2-13), 43.3 (CH_2-15), 43.4 (CH_2-19), 57.1 (CH-9), 67.2 (CH-12), 68.1 (C_q-11), 110.1 (C_q-3), 111.3 (CH-7), 118.8 (CH-4), 119.8 (CH-5), 122.5 (CH-6), 123.2 (CH-27), 125.3 (CH-21*), 125.5 (CH-22*), 125.9 (CH-25*), 126.4 (CH-26*), 127.3 (C_q-3a), 128.0 (CH-23), 128.9 (CH-24), 131.4 (C_q-27a), 133.8 (C_q-23a), 134.9 (C_q-2), 135.7 (C_q-7a**), 135.9 (C_q-20**), 171.1 ($C_{C=O}$-17) ppm (The signals with the same superscript (*,**) may be interchanged); **IR** (cm^{-1}): υmax = 1609 (C=O), 1457; **MS** (ES): *m/z* (%) = 423 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{27}H_{27}N_4O^+$ $[M + H]^+$: 423.2179; found: 423.2209.

(6*R*,13*R*,13a*S*)-13-((2-(1*H*-indol-3-yl)ethyl)amino)-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino[4,5-b]indol-5-one 262



Following the general procedure using 3 equivalents of tryptamine, **262** was obtained on purification by reversed-phase chromatography using a H_2O/ACN gradient (during 10 CV 10% ACN, over 20 CV to 50% ACN, during 10 CV 50% ACN) as a white powder. **Yield** 31% (66 mg); white powder ; m.p. 248-250 °C; $[\alpha]_D^{25}$ = -118.6 (c=0.41 in $CHCl_3$); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.63-1.80 (3H, m, C$\underline{H_2}$-13, C$\underline{H_a}H_b$-14), 1.81-1.92 (1H, m, $CH_a\underline{H_b}$-14), 2.28-2.35 (1H, m, N$\underline{H}$-18), 2.50-2.58 (1H, m, C$\underline{H_a}H_b$-19), 2.66 (1H, d, *J*=15.2 Hz, C$\underline{H_a}H_b$-20), 2.75-3.05 (5H, m, C$\underline{H_2}$-8, C$\underline{H_a}H_b$-15, $CH_a\underline{H_b}$-19, $CH_a\underline{H_b}$-20), 3.14 (1H, d, *J*=3.5 Hz, N$\underline{H}$-10), 3.35-3.41 (1H, m, C$\underline{H}$-12), 3.72-3.85 (1H, m, C$\underline{H}$-9), 6.88 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-5), 6.94 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-5'), 6.98-7.07 (3H, m, C$\underline{H}$-2', C$\underline{H}$-6, C$\underline{H}$-6'), 7.29 (1H, d, *J*=7.8 Hz, C$\underline{H}$-7'), 7.34 (2H, d, *J*=7.8 Hz, C$\underline{H}$-4', C$\underline{H}$-7), 7.47 (1H, d, *J*=7.8 Hz, C$\underline{H}$-4), 10.72 (1H, s, NH-1'), 10.77 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 21.7 (CH_2-14), 25.0 (CH_2-13, CH_2-20), 27.0 (CH_2-8), 43.3 (CH_2-15), 43.6 (CH_2-19), 56.6 (CH-9), 67.6 (CH-12), 68.0 (C_q-11), 108.1 (C_q-3'), 111.7 (CH-7'), 111.9 (CH-7), 113.2 (C_q-3), 118.2 (CH-4'), 118.5 (CH-5), 118.8 (CH-5'), 118.9 (CH-4), 121.3 (CH-6, CH-6'), 122.7 (CH-2'), 127.1 (C_q-3a'), 127.7 (C_q-3a), 136.3 (C_q-7a'), 136.7 (C_q-2, C_q-7a), 171.5 ($C_{C=O}$-17) ppm (The allocation of the signals CH_y-X and CH_y-X' may be interchanged); **IR** (cm^{-1}): υmax = 1622 (C=O), 1456; **MS** (ES): *m/z* (%) = 426 (100) $[M + H]^+$, 703 (30); **HRMS** (ESI): calcd. for $C_{26}H_{28}N_5O^+$ $[M + H]^+$: 426.2288; found: 426.2300.

(6*R*,13*S*,13a*S*)-13-allylthio-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 265



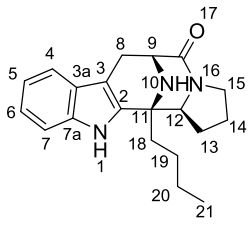
Using general procedure B using 3 equivalents of allyl mercaptan, **265** was obtained after purification by pTLC as a white powder. **Yield** 32% (55 mg); white powder; R_f=0.22 (EtOAc); m.p. 136-140 °C; $[\alpha]_D^{20}$ = -165.26 (c=0.38 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.63-1.76 (1H, m, C$\underline{H_a}H_b$-13), 1.76-1.87 (1H, m, C$\underline{H_a}H_b$-14), 1.91-1.99 (1H, m, $CH_a\underline{H_b}$-13), 1.99-2.07 (1H, m, $CH_a\underline{H_b}$-14), 2.45 (1H, s, N$\underline{H}$-10), 2.95 (1H, ddd, *J*=12.2 Hz, *J*=10.2 Hz, *J*=4.6 Hz, C$\underline{H_a}H_b$-15), 3.01 (1H, dd, *J*=16.0 Hz, *J*=1.3 Hz, C$\underline{H_a}H_b$-8), 3.02 (1H, ddt, *J*=13.6 Hz, *J*=7.4 Hz, *J*=1.2 Hz, C$\underline{H_a}H_b$-18), 3.16 (1H, dd, *J*=16.0 Hz, *J*=6.5 Hz, $CH_a\underline{H_b}$-8), 3.29 (1H, ddt, *J*=13.3 Hz, *J*=7.0 Hz, *J*=1.2 Hz, $CH_a\underline{H_b}$-18), 3.58 (1H, dd, *J*=11.9 Hz, *J*=4.7 Hz, C$\underline{H}$-12), 3.99 (1H, ddd, *J*=12.2 Hz, *J*=9.5 Hz, *J*=6.3 Hz, $CH_a\underline{H_b}$-15), 4.06 (1H, dd, *J*=6.5 Hz, *J*=1.3 Hz, C$\underline{H}$-9), 5.01 (1H, ddt, *J*=9.9 Hz, *J*=1.2 Hz, *J*=1.2 Hz, C$\underline{H_a}H_b$-20), 5.04 (1H, ddt, *J*=17.0 Hz, *J*=1.2 Hz, *J*=1.2 Hz, $CH_a\underline{H_b}$-20), 5.79 (1H, dddd, *J*=17.0 Hz, *J*=9.9 Hz, *J*=7.4 Hz, *J*=7.0 Hz, C$\underline{H}$-19), 7.13 (1H, ddd, *J*=7.8 Hz, *J*=7.8 Hz, *J*=1.1 Hz, C$\underline{H}$-5), 7.21 (1H, ddd, *J*=7.8 Hz, *J*=7.8 Hz, *J*=1.1 Hz, C$\underline{H}$-6), 7.36

(1H, d, *J*=7.8 Hz, CH-7), 7.48 (1H, d, *J*=7.8 Hz, CH-4), 8.16 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.7 (CH_2-14), 24.8 (CH_2-8), 26.2 (CH_2-13), 31.9 (CH_2-18), 43.1 (CH_2-15), 55.9 (CH-9), 63.1 (C_q-11), 67.0 (CH-12), 110.4 (C_q-3), 111.2 (CH-7), 117.9 (CH_2-20), 118.9 (CH-4), 120.0 (CH-5), 122.7 (CH-6), 127.2 (C_q-3a), 134.0 (CH-19), 134.3 (C_q-2), 135.5 (C_q-7a), 170.9 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3262 (NH), 1614 (C=O), 1448; **MS** (ES): *m/z* (%) = 340 (100) $[M + H]^+$, 679 (25); **HRMS** (ESI): calcd. for $C_{19}H_{22}N_3OS^+$ $[M + H]^+$: 340.1478; found: 340.1490.

7.4.4. Procedure for the synthesis of derivative 267

A solution of α-chloroamine **237b** (1 equiv., 0.5 mmol, 151 mg) in dry THF (10 mL) was cooled to -78 °C. Butyllithium (3 equiv, 1.5 mmol, 0.8 mL of 2 M BuLi in hexanes) was added and the mixture was allowed to warm to room temperature. After 30 minutes the reaction was quenched by the careful addition of water (10 mL). EtOAc (15 mL) was added subsequently and the layers were separated. The aqueous layer was extracted with EtOAc (10 mL). The combined organic phases were washed 3 times with water (10 mL) and dried over magnesium sulfate. Concentration of the solution under reduced pressure yielded a yellow powder. Purification by pTLC using EtOAc as eluent gave compound **267** as a white powder.

(6*R*,13*R*,13a*S*)-13-butyl-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 267

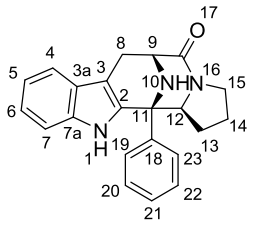


Yield 12% (19 mg); white powder; R_f=0.11 (EtOAc); m.p. 204-206 °C; $[\alpha]_D^{20}$ = -29.6 (c=0.27 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 0.85 (3H, t, *J*=7.1 Hz, CH_3-21), 1.05-1.17 (1H, m, CH_aH_b-19), 1.24-1.38 (3H, m, CH_aH_b-19, CH_2-20), 1.58-2.04 (7H, m, NH-10, CH_2-13, CH_2-14, CH_2-18), 2.87 (1H, ddd, *J*=12.1 Hz, *J*=9.8 Hz, *J*=4.4 Hz, CH_aH_b-15), 3.03 (1H, dd, *J*=16.1 Hz, *J*=1.7 Hz, CH_aH_b-8), 3.09 (1H, dd, *J*=16.1 Hz, *J*=6.0 Hz, CH_aH_b-8), 3.33 (1H, dd, *J*=11.6 Hz, *J*=4.5 Hz, CH-12), 3.98 (1H, ddd, *J*=12.1 Hz, *J*=9.5 Hz, *J*=6.0 Hz, CH_aH_b-15), 4.10 (1H, dd, *J*=6.0 Hz, *J*=1.7 Hz, CH-9), 7.09 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.1 Hz, CH-5), 7.15 (1H, ddd, *J*=7.7 Hz, *J*=7.7 Hz, *J*=1.1 Hz, CH-6), 7.32 (1H, d, *J*=7.7 Hz, CH-7), 7.46 (1H, d, *J*=7.7 Hz, CH-4), 8.26 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 14.1 (CH_3-21), 21.7 (CH_2-14), 23.3 (CH_2-20), 25.7 (CH_2-8), 25.8 (CH_2-19), 26.0 (CH_2-13), 35.0 (CH_2-18), 43.0 (CH_2-15), 53.1 (C_q-11), 55.0 (CH-9), 67.9 (CH-12), 108.5 (C_q-3), 111.1 (CH-7), 118.6 (CH-4), 119.8 (CH-5), 122.1 (CH-6), 127.3 (C_q-3a), 136.0 (C_q-7a), 136.9 (C_q-2), 171.2 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3254 (NH), 1614 (C=O), 1455; **MS** (ES): *m/z* (%) = 324 (100) $[M + H]^+$, 647 (85); **HRMS** (ESI): calcd. for $C_{20}H_{26}N_3O^+$ $[M + H]$+: 324.2070; found: 324.2082.

7.4.5. Procedure for the synthesis of derivative 270

A solution of α-chloroamine **237b** (1 equiv., 1.5 mmol, 453 mg) in dry THF (25 mL) was cooled to -78 °C. Phenyllithium (2 equiv., 3.0 mmol, 2.0 mL of 1.5 M PhLi in dibutyl ether) was added and the mixture was allowed to warm to room temperature. After 3 hours the reaction was quenched by the careful addition of ammonia chloride solution (20 mL). The layers were separated and the organic phase was washed another 2 times with ammonia chloride solution (20 mL) and dried over magnesium sulfate. Concentration under reduced pressure yielded an orange foam. Purification by reversed-phase chromatography using a H_2O/ACN gradient (during 2 CV 30% ACN, over 20 CV to 80% ACN) gave compound **270** as an orange powder.

(6*R*,13*R*,13a*S*)-13-phenyl-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 270



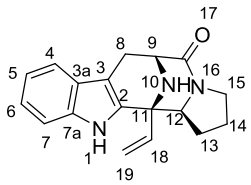
Yield 12% (62 mg); orange amorphous powder; m.p. >260 °C; $[\alpha]_D^{20}$ = -67 (c=0.2 in DMSO); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.34-1.47 (1H, m, C$\underline{H}_a$H$_b$-13), 1.56-1.65 (1H, m, CH$_a$$\underline{H}_b$-13), 1.70-1.81 (2H, m, C$\underline{H}_2$-14), 2.79 (1H, d, *J*=15.5 Hz, C$\underline{H}_a$H$_b$-8), 2.79-2.85 (1H, m, C$\underline{H}_a$H$_b$-15), 3.04 (1H, dd, *J*=15.5 Hz, *J*=6.5 Hz, CH$_a$$\underline{H}_b$-8), 3.80 (1H, d, *J*=3.8 Hz, N$\underline{H}$-10), 3.89-3.98 (2H, m, C$\underline{H}$-9, CH$_a$$\underline{H}_b$-15), 4.10 (1H, dd, *J*=11.6 Hz, *J*=4.8 Hz, C$\underline{H}$-12), 6.93 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, *J*=0.8 Hz, C$\underline{H}$-5), 7.00 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, *J*=0.8 Hz, C$\underline{H}$-6), 7.29-7.38 (3H,m, 1H, m, C$\underline{H}$-4, C$\underline{H}$-7, C$\underline{H}$-21), 7.45 (2H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-20, C$\underline{H}$-22), 7.81 (2H, d, *J*=7.5 Hz, C$\underline{H}$-19, C$\underline{H}$-23), 10.08 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 22.0 (CH_2-14), 25.4 (CH_2-8), 26.5 (CH_2-13), 43.4 (CH_2-15), 54.7 (C_q-11), 55.6 (CH-9), 67.2 (CH-12), 105.9 (C_q-3), 112.1 (CH-7), 118.1 (CH-4), 119.2 (CH-5), 121.5 (CH-6), 126.8 (C_q-3a), 126.9 (CH-19, CH-23), 127.7 (CH-21), 128.8 (CH-20, CH-22), 136.4 (C_q-7a), 137.9 (C_q-2), 142.0 (C_q-18), 171.4 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3274 (NH), 1633 (C=O), 1456; **MS** (ES): *m/z* (%) = 344 (100) $[M + H]^+$, 687 (85); **HRMS** (ESI): calcd. for $C_{22}H_{22}N_3O^+$ [M + H]+: 344.1757; found: 344.1765.

7.4.6. Procedure for the synthesis of derivative 274

To a solution of α-chloroamine **237b** (1 equiv., 1.0 mmol, 302 mg) in dry THF (15 mL), vinylmagnesium bromide (3 equiv., 3.0 mmol, 3.0 mL of 1.0M vinylmagnesium bromide in THF) was added. After one hour stirring at room temperature, the reaction was quenched by the careful addition of ammonia chloride solution (15 mL). The layers were separated and the organic phase was washed another two times with ammonia chloride solution (15 mL) and dried over magnesium sulfate. Concentration under reduced pressure yielded an brown oil. Purification by reversed-phase

chromatography using a H_2O/ACN gradient (during 10 CV 10% ACN, over 20 CV to 50% ACN) gave compound **274** as a yellow powder.

(6*R*,13*R*,13a*S*)-13-vinyl-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo[1',2':1,2]azocino-[4,5-b]indol-5-one 274



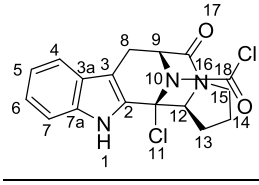
Yield 27% (80 mg); yellow amorphous powder; m.p. >260 °C; $[\alpha]_D^{25}$ = -56 (c=0.1 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.54-2.04 (5H, m, C$\underline{H_2}$-13, C$\underline{H_2}$-14, N$\underline{H}$-10), 2.86-2.95 (1H, m, C$\underline{H_a}H_b$-15), 3.10 (1H, d, *J*=2.3 Hz, C$\underline{H_a}H_b$-8), 3.11 (1H, d, *J*=5.9 Hz, C$H_a\underline{H_b}$-8), 3.54 (1H, dd, *J*=11.9 Hz, *J*=4.7 Hz, C$\underline{H}$-12), 4.02-4.10 (1H, m, C$H_a\underline{H_b}$-15), 4.19 (1H, dd, *J*=5.9 Hz, *J*=2.3 Hz, C$\underline{H}$-9), 5.43 (1H, d, *J*=10.9 Hz, C$\underline{H_a}H_b$-19), 5.51 (1H, d, *J*=17.3 Hz, C$H_a\underline{H_b}$-19), 6.30 (1H, dd, *J*=17.3 Hz, *J*=10.9 Hz, C$\underline{H}$-18), 7.11 (1H, dd, *J*=7.4 Hz, *J*=7.4 Hz, C$\underline{H}$-5), 7.18 (1H, dd, *J*=7.4 Hz, *J*=7.4 Hz, C$\underline{H}$-6), 7.32 (1H, d, *J*=7.4 Hz, C$\underline{H}$-7), 7.48 (1H, d, *J*=7.4 Hz, C$\underline{H}$-4), 7.97 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.7 (CH_2-14), 25.5 (CH_2-8), 26.8 (CH_2-13), 43.2 (CH_2-15), 54.3 (C_q-11), 54.9 (CH-9), 66.7 (CH-12), 107.9 (C_q-3), 111.0 (CH-7), 117.2 (CH_2-19), 118.7 (CH-4), 119.9 (CH-5), 122.4 (CH-6), 127.1 (C_q-3a), 135.8 (C_q-2*), 135.9 (C_q-7a*), 136.6 (CH-18), 170.6 ($C_{C=O}$-17) ppm (The signals with the same superscript (*,**) may be interchanged); **IR** (cm^{-1}): υmax = 3155 (NH), 1613 (C=O), 1467; **MS** (ES): *m/z* (%) = 294 (100) $[M + H]^+$, 587 (65); **HRMS** (ESI): calcd. for $C_{18}H_{20}N_3O^+$ [M + H]+: 294.1601; found: 294.1610.

7.5. Carbamate and urea derivatives of the α-chloroamine 327b

7.5.1. Synthesis of the carbamoyl chloride 277

Diketopiperazine **2b** (1 equiv., 3.0 mmol, 0.85 g) was suspended in dry CH_2Cl_2 (50 mL) and cooled with an ice-bath to 0 °C under a nitrogen atmosphere. Diphosgene (3 equiv., 9.0 mmol, 1.1 mL), dissolved in dry CH_2Cl_2 (30 mL), was added dropwise to the suspension. The mixture was heated to reflux. The reaction was monitored using HPLC-MS and after complete conversion (ca. 36 h) of the intermediate α-chloroamine **237b**, the organic phase was washed with saturated $NaHCO_3$ solution and with water. The organic phase was dried over magnesium sulfate and concentrated *in vacuo* to yield the crude carbamoyl chloride **277** as a brown foam.

(6*R*,13*S*,13a*S*)-13-chloro-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carbonyl chloride 277

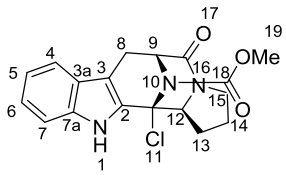


Crude yield 90% (0.98 g, ±90% pure); brown foam; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.77-1.91 (1H, m, C$\underline{H_a}H_b$-14), 1.95-2.10 (2H, m, C$\underline{H_a}H_b$-13, C$H_a\underline{H_b}$-14), 2.22-2.31 (1H, m, C$H_a\underline{H_b}$-13), 3.00-3.08 (1H, m, C$\underline{H_a}H_b$-15), 3.21 (1H, d, *J*=16.4 Hz,

C$\underline{H}_a$H$_b$-8), 3.39 (1H, dd, *J*=16.4 Hz, *J*=6.0 Hz, CH$_a\underline{H}_b$-8), 3.87 (1H, dd, *J*=11.2 Hz, *J*=4.7 Hz, C$\underline{H}$-12), 3.97-4.06 (1H, m, CH$_a\underline{H}_b$-15), 5.65 (1H, d, *J*=6.0 Hz, C$\underline{H}$-9), 7.17 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.27 (1H, dd, *J*=7.5 Hz, *J*=7.5 Hz, C$\underline{H}$-5), 7.38 (1H, d, *J*=7.5 Hz, C$\underline{H}$-7), 7.49 (1H, d, *J*=7.5 Hz, C$\underline{H}$-4), 8.37 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, CDCl$_3$) δ** 21.2 (CH_2-14), 24.0 (CH_2-8), 28.4 (CH_2-13), 44.6 (CH_2-15), 64.2 (CH-9), 69.9 (CH-12), 76.4 (C_q-11), 109.2 (C_q-3), 111.8 (CH-7), 119.4 (CH-4), 120.9 (CH-5), 124.3 (CH-6), 125.8 (C_q-3a), 131.7 (C_q-2), 136.3 (C_q-7a), 146.7 ($C_{C=O}$-18), 166.1 ($C_{C=O}$-17) ppm.

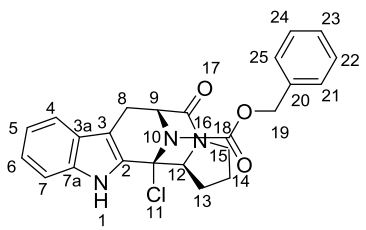
7.5.2. Carbamate derivatives

methyl (6*R*,13*S*,13a*S*)-13-chloro-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxylate 278



Following the general procedure 7.5.1, the crude carbamoyl chloride **277** was dissolved in a minimal amount of methanol and colourless crystals of **278** were formed, which turned white after drying to air. Fine fibrous crystals were formed. **Yield** 65% (0.70 g); colourless, fibrous crystals; m.p. 166-168 °C; $[\alpha]_D^{25}$ = -74.5 (c=0.51 in $CHCl_3$); **^{1}H NMR (400 MHz, CDCl$_3$) δ** 1.73-1.89 (1H, m, C$\underline{H}_a$H$_b$-14), 1.94-2.09 (2H, m, C$\underline{H}_a$H$_b$-13, CH$_a\underline{H}_b$-14), 2.18-2.28 (1H, m, CH$_a\underline{H}_b$-13), 2.96-3.05 (1H, m, C$\underline{H}_a$H$_b$-15), 3.12 (1H, dd, *J*=16.3 Hz, *J*=1.3 Hz, C$\underline{H}_a$H$_b$-8), 3.29 (1H, dd, *J*=16.3 Hz, *J*=6.2 Hz, CH$_a\underline{H}_b$-8), 3.77 (3H, s, C$\underline{H}_3$-19), 3.79-3.85 (1H, m, C$\underline{H}$-12), 3.97-4.06 (1H, m, CH$_a\underline{H}_b$-15), 5.48 (1H, dd, *J*=6.2 Hz, *J*=1.3 Hz, C$\underline{H}$-9), 7.14 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, C$\underline{H}$-5), 7.24 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, C$\underline{H}$-6), 7.36 (1H, d, *J*=8.0 Hz, C$\underline{H}$-7), 7.48 (1H, d, *J*=8.0 Hz, C$\underline{H}$-4), 8.38 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, CDCl$_3$) δ** 21.3 (CH_2-14), 23.8 (CH_2-8), 28.4 (CH_2-13), 44.4 (CH_2-15), 53.3 (CH_3-19), 60.5 (CH-9), 70.1 (CH 12), 74.9 (C_q-11), 109.5 (C_q-3), 111.6 (CH-7), 119.3 (CH-4), 120.6 (CH-5), 123.9 (CH-6), 126.3 (C_q-3a), 133.2 (C_q-2), 136.1 (C_q-7a), 155.1 ($C_{C=O}$-18), 167.7 ($C_{C=O}$-17) ppm. **IR** (cm^{-1}): υmax = 3177 (NH), 1722 (C=O), 1620 (C=O), 1439; **MS** (ES): *m/z* (%) = 360/362 (100/33) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{18}H_{19}ClN_3O_3^+$ $[M + H]^+$: 360.1109; found: 360.1112.

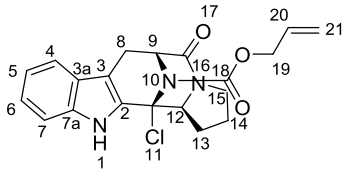
benzyl (6*R*,13*S*,13a*S*)-13-chloro-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxylate 279



Following general procedure 7.5.1 on a 2.0 mmol scale, the crude carbamoyl chloride **277** was dissolved in benzyl alcohol (3 equiv., 6.0 mmol, 0.62 mL). After 4 days the product was isolated as a white powder with reversed-phase chromatography using a H_2O/ACN gradient (during 2 CV 30% ACN, over 20 CV to 80% ACN).**Yield** 36% (0.31 g); colourless crystals; m.p. 144-150 °C; $[\alpha]_D^{25}$ = -54.5 (c=0.35 in $CHCl_3$); **^{1}H NMR (400 MHz,**

$CDCl_3$) δ 1.74-1.88 (1H, m, C$\underline{H}_a$$H_b$-14), 1.90-2.05 (2H, m, C$\underline{H}_a$$H_b$-13, $CH_a\underline{H}_b$-14), 2.18-2.26 (1H, m, $CH_a\underline{H}_b$-13), 2.96-3.04 (1H, m, C$\underline{H}_a$$H_b$-15), 3.10 (1H, dd, *J*=16.3 Hz, *J*=1.2 Hz, C$\underline{H}_a$$H_b$-8), 3.26 (1H, dd, *J*=16.3 Hz, *J*=6.2 Hz, $CH_a\underline{H}_b$-8), 3.81 (1H, dd, *J*=11.1 Hz, *J*=4.9 Hz, C$\underline{H}$-12), 3.94-4.03 (1H, m, $CH_a\underline{H}_b$-15), 5.15 (1H, d, *J*=12.2 Hz, C$\underline{H}_a$$H_b$-19), 5.22 (1H, d, *J*=12.2 Hz, $CH_a\underline{H}_b$-19), 5.52 (1H, dd, *J*=6.2 Hz, *J*=1.2 Hz, C$\underline{H}$-9), 7.14 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C$\underline{H}$-5), 7.22-7.28 (1H, m, C$\underline{H}$-6), 7.30-7.39 (6H, m, C$\underline{H}$-7, C$\underline{H}$-21, C$\underline{H}$-22, C$\underline{H}$-23, C$\underline{H}$-24, C$\underline{H}$-25), 7.47 (1H, d, *J*=7.6 Hz, C$\underline{H}$-4), 8.19 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.3 (CH_2-14), 23.8 (CH_2-8), 28.4 (CH_2-13), 44.4 (CH_2-15), 60.5 (CH-9), 68.2 (CH_2-19), 70.2 (CH-12), 75.1 (C_q-11), 109.5 (C_q-3), 111.6 (CH-7), 119.3 (CH-4), 120.6 (CH-5), 123.9 (CH-6), 126.3 (C_q-3a), 128.3 (CH-21, CH-25), 128.4 (CH-23), 128.6 (CH-22, CH-24), 133.1 (C_q-2), 135.5 (C_q-20), 136.1 (C_q-7a), 154.5 ($C_{C=O}$-18), 167.6 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3264 (NH), 1725 (C=O), 1636 (C=O), 1451; **MS** (ES): *m/z* (%) = 436/438 (100/30) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{24}H_{23}ClN_3O_3^+$ $[M + H]^+$: 436.1422; found: 436.1418.

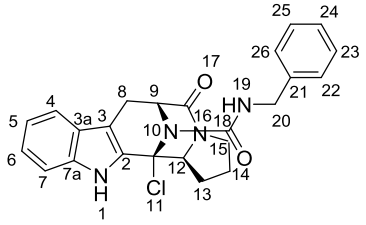
allyl (6*R*,13*S*,13a*S*)-13-chloro-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxylate 280



Following general procedure 7.5.1 on a 2.0 mmol scale, the crude carbamoyl chloride **277** was dissolved in allyl alcohol (3 equiv., 6.0 mmol, 0.41 mL). After 4 days the product **280** was isolated as a yellow powder with column chromatography using a mixture of EtOAc and petroleum ether as eluent. **Yield** 44% (0.34 g); yellow powder; R_f=0.15 (2/8 petroleum ether/EtOAc); m.p. 107-109 °C; $[\alpha]_D^{25}$ = -67.7 (c=0.64 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.73-1.90 (1H, m, C$\underline{H}_a$$H_b$-14), 1.94-2.10 (2H, m, C$\underline{H}_a$$H_b$-13, $CH_a\underline{H}_b$-14), 2.18-2.29 (1H, m, $CH_a\underline{H}_b$-13), 2.96-3.05 (1H, m, C$\underline{H}_a$$H_b$-15), 3.13 (1H, dd, *J*=16.3 Hz, *J*=1.2 Hz, C$\underline{H}_a$$H_b$-8), 3.31 (1H, dd, *J*=16.3 Hz, *J*=6.2 Hz, $CH_a\underline{H}_b$-8), 3.82 (1H, dd, *J*=11.1 Hz, *J*=4.9 Hz, C$\underline{H}$-12), 3.93-4.06 (1H, m, $CH_a\underline{H}_b$-15), 4.59-4.73 (2H, m, C$\underline{H}_2$-19), 5.26 (1H, dd, *J*=10.4 Hz, *J*=1.3 Hz, C$\underline{H}_a$$H_b$-21), 5.34 (1H, dd, *J*=17.2 Hz, *J*=1.3 Hz, $CH_a\underline{H}_b$-21), 5.52 (1H, dd, *J*=6.2 Hz, *J*=1.2 Hz, C$\underline{H}$-9), 5.88-6.00 (1H, m, C$\underline{H}$-20), 7.14 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.24 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.36 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7), 7.48 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 8.37 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.3 (CH_2-14), 23.8 (CH_2-8), 28.4 (CH_2-13), 44.4 (CH_2-15), 60.5 (CH-9), 67.1 (CH_2-19), 70.2 (CH-12), 75.0 (C_q-11), 109.4 (C_q-3), 111.6 (CH-7), 118.7 (CH_2-21), 119.3 (CH-4), 120.6 (CH-5), 123.8 (CH-6), 126.2 (C_q-3a), 131.9 (CH-20), 133.2 (C_q-2), 136.2 (C_q-7a), 154.3 ($C_{C=O}$-18), 167.7 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3265 (NH), 1728 (C=O), 1634 (C=O), 1451; **MS** (ES): *m/z* (%) = 386/388 (100/35) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{20}H_{21}ClN_3O_3^+$ $[M + H]^+$: 386.1266; found: 386.1276.

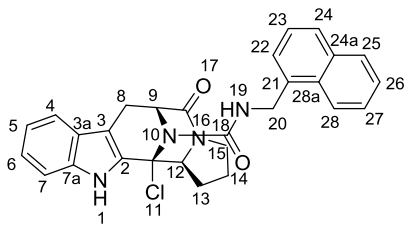
7.5.3. Urea derivatives

(6*R*,13*S*,13a*S*)-*N*-benzyl-13-chloro-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxamide 281



Following general procedure 7.5.1 on a 2.0 mmol scale, benzylamine (3 equiv., 6.0 mmol, 0.66 mL) was added to the crude carbamoyl chloride **277**. A brown precipitate is formed and the product **281** was isolated as a brown foam with column chromatography using a mixture of EtOAc and petroleum ether as eluent. **Yield** 58% (0.34 g); brown foam; R_f=0.40 (2/8 petroleum ether/EtOAc); m.p. >260 °C; $[\alpha]_D^{25}$ = -344.0 (c=0.53 in DMSO); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.72-1.85 (1H, m, CH_aH_b-14), 1.93-2.05 (2H, m, CH_aH_b-13, CH_aH_b-14), 2.08-2.16 (1H, m, CH_aH_b-13), 2.95-3.03 (1H, m, CH_aH_b-15), 3.08 (1H, dd, *J*=16.4 Hz, *J*=1.1 Hz, CH_aH_b-8), 3.28 (1H, dd, *J*=16.4 Hz, *J*=6.4 Hz, CH_aH_b-8), 3.83 (1H, dd, *J*=11.1 Hz, *J*=4.9 Hz, CH-12), 4.01-4.10 (1H, m, CH_aH_b-15), 4.40 (1H, dd, *J*=14.7Hz, *J*=5.4 Hz, CH_aH_b-20), 4.50 (1H, dd, *J*=14.7Hz, *J*=5.4 Hz, CH_aH_b-20), 5.18 (1H, dd, *J*=6.4 Hz, *J*=1.1 Hz, CH-9), 6.06 (1H, t, *J*=5.4 Hz, NH-19), 7.14 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, CH-5), 7.22-7.38 (7H, m, CH-6, CH-7, CH-22, CH-23, CH-24, CH-25, CH-26), 7.48 (1H, d, *J*=7.6 Hz, CH-4), 8.17 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.4 (CH_2-14), 23.7 (CH_2-8), 28.4 (CH_2-13), 44.4 (CH_2-15), 45.3 (CH_2-20), 61.5 (CH-9), 70.1 (CH-12), 78.4 (C_q-11), 110.3 (C_q-3), 111.5 (CH-7), 119.5 (CH-4), 120.6 (CH-5), 123.8 (CH-6), 126.4 (C_q-3a), 127.6 (CH-24), 127.8 (CH-22, CH-26), 128.8 (CH-23, CH-25), 132.8 (C_q-2), 136.2 (C_q-7a), 137.9 (C_q-21), 158.3 ($C_{C=O}$-18), 168.2 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3174 (NH), 1609 (C=O), 1457; **MS** (ES): *m/z* (%) = 435/437 (15/5) $[M + H]^+$, 302/304 (25/8), 241 (100); **HRMS** (ESI): calcd. for $C_{24}H_{24}ClN_4O_2^+$ $[M + H]^+$: 435.1582; found: 435.1591.

(6*R*,13*S*,13a*S*)-13-chloro-*N*-(naphthalen-1-ylmethyl)-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo[1',2':1,2]azocino[4,5-b]indole-14-carboxamide 282

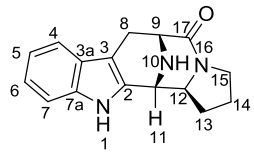


Following general procedure 7.5.1 on a 4.0 mmol scale, 1-napthylmethylamine (3 equiv., 12.0 mmol, 1.8 mL) was added to the crude carbamoyl chloride **277**. A brown precipitate is formed. Compound **282** was obtained as a white powder after rinsing the product with methanol. **Yield** 32% (0.62 g); white powder; m.p. >260 °C; $[\alpha]_D^{25}$ = -248.1 (c=0.83 in DMSO); **^{1}H NMR (400 MHz, DMSO-d6) δ** 1.70-1.87 (2H, m, CH_2-14), 1.95-2.10 (2H, m, CH_2-13), 2.77 (1H, d, *J*=16.1 Hz, CH_aH_b-8), 2.89-2.98 (1H, m, CH_aH_b-15), 3.04 (1H, dd, *J*=16.1 Hz, *J*=6.2 Hz, CH_aH_b-8), 3.76-3.87 (2H, m, CH-12, CH_aH_b-15), 4.62-4.82 (3H, m, CH-9, CH_2-20), 7.01 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, CH-5), 7.14 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, CH-6), 7.39 (1H, d, *J*=7.8 Hz, CH-7), 7.42 (1H, d, *J*=7.8 Hz, CH-4), 7.45-7.50 (2H, m, CH-22, CH-23), 7.53-7.57

(2H, m, CH-26, CH-27), 7.87 (1H, dd, *J*=7.3 Hz, *J*=1.7 Hz, CH-24), 7.95-7.98 (1H, m, CH-25), 8.09-8.13 (1H, m, CH-28), 8.25 (1H, t, *J*=5.7 Hz, NH-19), 11.24 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, DMSO-d6) δ** 21.3 (CH_2-14), 23.6 (CH_2-8), 28.5 (CH_2-13), 42.2 (CH_2-20), 44.4 (CH_2-15), 63.0 (CH-9), 69.6 (CH-12), 76.5 (C_q-11), 107.4 (C_q-3), 112.3 (CH-7), 118.9 (CH-4), 119.7 (CH-5), 122.7 (CH-6), 124.0 (CH-28), 125.8 (CH-22*), 126.1 (C_q-3a), 126.2 (CH-23*), 126.3 (CH-26), 126.6 (CH-27), 128.1 (CH-24), 129.0 (CH-25), 131.4 (C_q-28a), 133.8 (C_q-24a), 134.7 (C_q-2), 135.1 (C_q-21), 136.5 (C_q-7a), 158.6 ($C_{C=O}$-18), 167.9 ($C_{C=O}$-17) ppm (The signals with the same superscript (*) may be interchanged); **IR** (cm^{-1}): υmax = 3172 (NH), 1665 (C=O), 1630 (C=O), 1505, 1456; **MS** (ES): *m/z* (%) = 485/487 (10/3) $[M + H]^+$, 302/304 (30/10), 141 (100); **HRMS** (ESI): calcd. for $C_{28}H_{26}ClN_4O_2^+$ $[M + H]^+$: 485.1739; found: 485.1744.

7.5.4. Hydrogenolysis of 279

To a solution of compound **279** (0.089 mmol, 39 mg) in MeOH, 10 wt% of Pd/C (4 mg) was added. The reaction mixture was stirred under 5 atm of H_2 for 4 hours at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. The filtrate was concentrated *in vacuo* to give the crude product **284**. The pure product **284** was obtained after pHPLC using an isocratic gradient of H_2O/ACN (20% ACN).



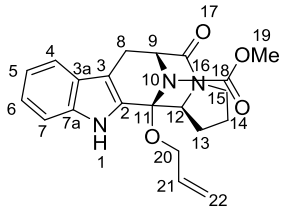
Yield 17% (4 mg); white powder; **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.78-2.06 (5H, m, CH_2-13, CH_2-14, NH-10), 2.91-2.99 (1H, m, CH_aH_b-15), 3.05 (1H, d, *J*=16.3 Hz, CH_aH_b-8), 3.14 (1H, dd, *J*=16.3 Hz, *J*=6.6 Hz, CH_aH_b-8), 3.44 (1H, dd, *J*=11.2 Hz, *J*=5.1 Hz, CH-12), 4.00-4.09 (1H, m, CH_aH_b-15), 4.10 (1H, d, *J*=6.6 Hz, CH-9), 4.34 (1H, s, CH-11), 7.11 (1H, dd, *J*=7.3 Hz, *J*=7.3 Hz, CH-5), 7.18 (1H, dd, *J*=7.3 Hz, *J*=7.3 Hz, CH-6), 7.33 (1H, d, *J*=7.3 Hz, CH-7), 7.49 (1H, d, *J*=7.3 Hz, CH-4), 7.87 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 22.1 (CH_2-14), 25.1 (CH_2-8), 28.4 (CH_2-13), 43.0 (CH_2-15), 46.9 (CH-11), 53.9 (CH-9), 63.7 (CH-12), 107.0 (C_q-3), 111.0 (CH-7), 118.5 (CH-4), 120.0 (CH-5), 122.3 (CH-6), 127.1 (C_q-3a), 134.6 (C_q-2), 135.6 (C_q-7a), 171.0 ($C_{C=O}$-17) ppm; **MS** (ES): *m/z* (%) = 286 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{16}H_{18}N_3O^+$ $[M + H]^+$: 286.1444; found: 286.1445.

7.5.5. Substitution of chlorine in carbamate 278

The nucleophile (1.5 equiv., 0.75 mmol) was dissolved in THF (5 mL) at room temperature. The solution was cooled to 0 °C and sodium hydride (1.5 equiv., 0.75 mmol, 30 mg, 60% in mineral oil) was added. After stirring for 15 minutes at 0 °C, carbamate **278** (1 equiv., 0.5 mmol, 180 mg) was added to the mixture. The mixture was allowed to warm up to room temperature and was kept stirring until the conversion was complete. The reaction was monitored using HPLC-MS. An ammonia chloride solution (10 mL) and EtOAc (15 mL) were added subsequently. The layers were separated

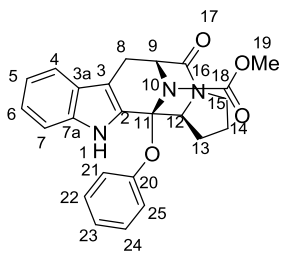
and the aqueous phase was extracted with 10 mL EtOAc. The combined organic phases were washed 3 times with water (10 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to remove the solvent. When necessary, further purification was done by reversed-phase chromatography using a gradient of water and acetonitrile as eluent or recrystallization to provide the desired compound.

methyl (6*R*,13*S*,13a*S*)-13-(allyloxy)-5-oxo-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxylate 286



Following general procedure 7.5.5 using 1.5 equivalents of allyl alcohol, **286** was obtained after purification by reversed-phase chromatography (2 CVs 30% ACN, over 20 CVs to 80% ACN) as a white powder. **Yield** 9% (17 mg); white powder; m.p. 152-156 °C; $[\alpha]_D^{25}$ = -30.5 (c=0.16 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.70-1.86 (2H, m, C$\underline{H}_a$H$_b$-13, C$\underline{H}_a$H$_b$-14), 1.94-2.05 (1H, m, CH$_a\underline{H}_b$-14), 2.10-2.19 (1H, m, CH$_a\underline{H}_b$-13), 2.92-3.01 (1H, m, C$\underline{H}_a$H$_b$-15), 3.14 (1H, dd, *J*=16.1 Hz, *J*=1.8 Hz, C$\underline{H}_a$H$_b$-8), 3.22 (1H, dd, *J*=16.1 Hz, *J*=5.8 Hz, CH$_a\underline{H}_b$-8), 3.66 (1H, dd, *J*=11.3 Hz, *J*=4.8 Hz, C$\underline{H}$-12), 3.76 (3H, s, C$\underline{H}_3$-19), 3.85-3.96 (2H, m, CH$_a\underline{H}_b$-15, C$\underline{H}_a$H$_b$-20), 4.25-4.32 (1H, m, CH$_a\underline{H}_b$-20), 5.23 (1H, dd, *J*=10.5 Hz, *J*=1.6 Hz, C$\underline{H}_a$H$_b$-22), 5.34 (1H, dd, *J*=17.3 Hz, *J*=1.6 Hz, CH$_a\underline{H}_b$-22), 5.50 (1H, dd, *J*=5.8 Hz, *J*=1.8 Hz, C$\underline{H}$-9), 5.94-6.05 (1H, m, C$\underline{H}$-21), 7.14 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-5), 7.24 (1H, dd, *J*=7.8 Hz, *J*=7.8 Hz, C$\underline{H}$-6), 7.35 (1H, d, *J*=7.8 Hz, C$\underline{H}$-7), 7.51 (1H, d, *J*=7.8 Hz, C$\underline{H}$-4), 8.10 (1H, s, N$\underline{H}$-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.7 (CH_2-14), 23.9 (CH_2-8), 26.2 (CH_2-13), 43.6 (CH_2-15), 53.0 (CH_3-19), 59.7 (CH-9), 66.5 (CH_2-20), 67.9 (CH-12), 87.0 (C_q-11), 111.4 (CH-7), 111.7 (C_q-3), 116.4 (CH_2-22), 119.2 (CH-4), 120.3 (CH-5), 123.3 (CH-6), 126.3 (C_q-3a), 132.5 (C_q-2), 134.3 (CH-21), 136.2 (C_q-7a), 155.6 ($C_{C=O}$-18), 168.8 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3258 (NH), 1692 (C=O), 1634 (C=O), 1439; **MS** (ES): *m/z* (%) = 382 (100) $[M + H]^+$, 763 (40); **HRMS** (ESI): calcd. for $C_{21}H_{24}N_3O_4^+$ $[M + H]^+$: 382.1761; found: 382.1761.

methyl (6*R*,13*S*,13a*S*)-5-oxo-13-phenoxy-2,3,5,6,7,12,13,13a-octahydro-1*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indole-14-carboxylate 287



Following general procedure 7.5.5 using 1.5 equivalents of phenol, **287** was obtained after recrystallization in methanol. **Yield** 38% (79 mg); colourless crystals; m.p. 221-223 °C; $[\alpha]_D^{25}$ = +14.8 (c=0.36 in $CHCl_3$); **^{1}H NMR (400 MHz, $CDCl_3$) δ** 1.76-2.11 (3H, m, C$\underline{H}_a$H$_b$-13, C$\underline{H}_2$-14), 2.28-2.36 (1H, m, CH$_a\underline{H}_b$-13), 2.97-3.05 (1H, m, C$\underline{H}_a$H$_b$-15), 3.26 (1H, dd, *J*=16.3 Hz, *J*=1.4 Hz, C$\underline{H}_a$H$_b$-8), 3.38 (1H, dd, *J*=16.3 Hz, *J*=6.1 Hz, CH$_a\underline{H}_b$-8), 3.51 (3H, s, C$\underline{H}_3$-19),

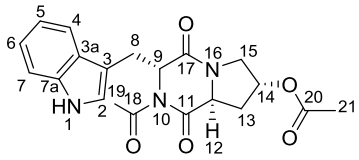
3.82 (1H, dd, *J*=11.4 Hz, *J*=4.9 Hz, CH-12), 3.92-4.01 (1H, m, CH_aH_b-15), 5.61 (1H, dd, *J*=6.1 Hz, *J*=1.4 Hz, CH-9), 6.81-6.94 (3H, m, CH-21, CH-23, CH-25), 7.08-7.24 (5H, m, CH-5, CH-6, CH-7, CH-22, CH-24), 7.53 (1H, d, *J*=7.6 Hz, CH-4), 7.80 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 21.6 (CH_2-14), 23.8 (CH_2-8), 26.6 (CH_2-13), 43.7 (CH_2-15), 53.0 (CH_3-19), 59.7 (CH-9), 68.5 (CH-12), 85.8 (C_q-11), 111.0 (C_q-3), 111.7 (CH-7), 116.4 (CH-21, CH-25), 119.2 (CH-4), 120.4 (CH-5), 122.1 (CH-23), 123.3 (CH-6), 126.3 (C_q-3a), 129.4 (CH-22, CH-24), 132.0 (C_q-2), 136.6 (C_q-7a), 155.1 ($C_{C=O}$-18, C_q-20), 168.4 ($C_{C=O}$-17) ppm; **IR** (cm^{-1}): υmax = 3058 (NH), 1698 (C=O), 1627 (C=O), 1439; **MS** (ES): *m/z* (%) = 418 (100) $[M + H]^+$, 834 (80); **HRMS** (ESI): calcd. for $C_{24}H_{24}N_3O_4^+$ $[M + H]^+$: 418.1761; found: 418.1794.

7.6. Introducing the α-chloroamine in cyclo(D-Trp, L-Hyp) 190b

7.6.1. Introduction of acetyl groups on cyclo(D-Trp, L-Hyp) 190b

Diketopiperazine cyclo(D-Trp, L-Hyp) **190b** (1 equiv., 1.0 mmol, 0.30 g) was dissolved in dry CH_2Cl_2 (20 mL) under a nitrogen atmosphere. Triethylamine (1.1 equiv., 1.1 mmol, 0.15 mL), DMAP (0.05 equiv., 0.05 mmol, 6 mg) and Ac_2O (1.1 equiv., 1.1 mmol, 0.10 mL) were added to the solution. When the conversion stagnated, a supplementary amount of Ac_2O (1.1 equiv., 1.1 mmol, 0.10 mL) was added and the mixture was left to stir overnight. Next, the solution was washed three times with 1 M HCl (3×15 mL), saturated aq. $NaHCO_3$ (3×15 mL) and water (15 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo* yielding a mixture of di- and tri-acetylated diketopiperazine. Both compounds were isolated using reversed-phase chromatography (2 CVs 20% ACN, over 20 CVs to 60% ACN).

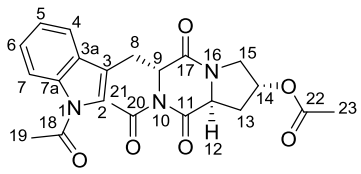
(3*R*,7*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-2-acetyl-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-7-yl acetate 293



Yield 34% (0.13 g); white foam; m.p. 198-204 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.76-1.83 (1H, m, CH_aH_b-13), 1.83 (3H, s, CH_3-21), 2.04 (1H, dd, *J*=13.8 Hz, *J*=5.9 Hz, CH_aH_b-13), 2.50 (1H, dd, *J*=11.7 Hz, *J*=5.9 Hz, CH-12), 2.60 (3H, s, CH_3-19), 3.08 (1H, d, *J*=14.0 Hz, CH_aH_b-15), 3.35 (1H, dd, *J*=15.0 Hz, *J*=5.3 Hz, CH_aH_b-8), 3.63 (1H, dd, *J*=15.0 Hz, *J*=2.9 Hz, CH_aH_b-8), 3.76 (1H, dd, *J*=14.0 Hz, *J*=5.1 Hz, CH_aH_b-15), 5.03 (1H, dd, *J*=5.1 Hz, *J*=5.1 Hz, CH-14), 5.31 (1H, dd, *J*=5.3 Hz, *J*=2.9 Hz, CH-9), 6.91 (1H, s, CH-2), 7.12 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, CH-5), 7.18 (1H, dd, *J*=8.0 Hz, *J*=8.0 Hz, CH-6), 7.37 (1H, d, *J*=8.0 Hz, CH-7), 7.57 (1H, d, *J*=8.0 Hz, CH-4), 8.20 (1H, s, NH-1) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 20.9 (CH_3-21), 27.8 (CH_3-19), 28.0 (CH_2-8), 35.9 (CH_2-13), 51.7 (CH_2-15), 57.4 (CH-12), 59.7 (CH-9), 69.8 (CH-14), 109.8 (C_q-3), 111.1 (CH-7), 119.2 (CH-4), 120.1 (CH-5), 122.8 (CH-6), 124.2 (CH-2), 127.2 (C_q-3a), 136.1 (C_q-7a), 165.1 ($C_{C=O}$-17), 169.8 ($C_{C=O}$-11, $C_{C=O}$-20), 171.9

($C_{C=O}$-18) ppm; **IR** (cm^{-1}): υmax = 3299 (NH), 1737 (C=O), 1710 (C=O), 1660 (C=O); **MS** (ES): *m/z* (%) = 384 (100) $[M + H]^+$, 766 (20); **HRMS** (ESI): calcd. for $C_{20}H_{22}N_3O_5^+$ $[M + H]^+$: 384.1554; found: 384.1548.

(3*R*,7*R*,8a*S*)-2-acetyl-3-((1-acetyl-1*H*-indol-3-yl)methyl)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-7-yl acetate 294



Yield 18% (0.076 g); white powder; m.p. 90-96 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.87 (3H, s, C$\underline{H}_3$-23), 1.87-1.95 (1H, m, C$\underline{H}_a$H$_b$-13), 2.16 (1H, dd, *J*=13.9 Hz, *J*=5.8 Hz, CH$_a$$\underline{H}_b$-13), 2.60 (6H, s, C$\underline{H}_3$-19, C$\underline{H}_3$-21), 3.00 (1H, dd, *J*=11.5 Hz, *J*=5.9 Hz, C$\underline{H}$-12), 3.17 (1H, d, *J*=14.0 Hz, C$\underline{H}_a$H$_b$-15), 3.33 (1H, dd, *J*=15.0 Hz, *J*=5.5 Hz, C$\underline{H}_a$H$_b$-8), 3.50 (1H, dd, *J*=15.0 Hz, *J*=3.4 Hz, CH$_a$$\underline{H}_b$-8), 3.78 (1H, dd, *J*=14.0 Hz, *J*=5.0 Hz, CH$_a$$\underline{H}_b$-15), 5.12 (1H, dd, *J*=5.0 Hz, *J*=5.0 Hz, C$\underline{H}$-14), 5.33 (1H, dd, *J*=5.5 Hz, *J*=3.4 Hz, C$\underline{H}$-9), 7.20 (1H, s, C$\underline{H}$-2), 7.29 (1H, dd, *J*=7.4 Hz, *J*=7.4 Hz, C$\underline{H}$-5), 7.36 (1H, dd, *J*=7.4 Hz, *J*=7.4 Hz, C$\underline{H}$-6), 7.49 (1H, d, *J*=7.4 Hz, C$\underline{H}$-4), 8.43 (1H, d, *J*=7.4 Hz, C$\underline{H}$-7) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 20.9 (CH_3-23), 24.1 (CH_3-19), 27.8 (CH_2-8, CH_3-21), 36.0 (CH_2-13), 51.7 (CH_2-15), 57.6 (CH-12), 59.1 (CH-9), 69.8 (CH-14), 116.3 (C_q-3), 116.7 (CH-7), 119.0 (CH-4), 123.9 (CH-5), 124.8 (CH-2), 126.0 (CH-6), 129.9 (C_q-3a), 135.7 (C_q-7a), 164.6 ($C_{C=O}$-17), 168.4 ($C_{C=O}$-18), 169.3 ($C_{C=O}$-11), 169.8 ($C_{C=O}$-22), 171.9 ($C_{C=O}$-20) ppm; **IR** (cm^{-1}): υmax = 1738 (C=O), 1704 (C=O), 1668 (C=O), 1452; **MS** (ES): *m/z* (%) = 426 (100) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{22}H_{24}N_3O_6^+$ $[M + H]^+$: 424.1509; found: 424.1533.

7.6.2. Introduction of Boc groups on dipeptide 182b

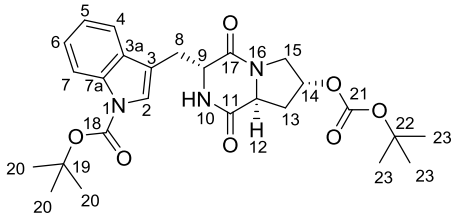
Dipeptide **182b** (1 equiv., 8.0 mmol, 3.7 g), synthesized according to the general procedure in 4.2.1, was dissolved in dry ACN (50 mL) under a nitrogen atmosphere. DMAP (0.2 equiv, 1.6 mmol, 0.20 g) and Boc_2O (1.2 equiv., 9.6 mmol, 2.10 g) were added to the solution. To obtain the fully diprotected product, a supplementary amount of Boc_2O (1 equiv., 8.0 mmol, 1.75 g) was added and the mixture was left to stir overnight. Next, the mixture was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL). The organic layer was washed three times with brine (3×15 mL) and subsequently dried over magnesium sulfate. Evaporation of the solvent under reduced pressure provided the crude dipeptide **296** (4.95 g).

7.6.3. Hydrogenolysis and cyclization of Boc-protected dipeptide 296

To a solution of dipeptide **296** in MeOH (100 mL), 5 wt% of Pd/C (0.25 g) was added. The reaction mixture was stirred under 5 atm of H_2 overnight at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. The methanolic solution was stirred at room temperature until ring closure was complete, which was monitored with HPLC-MS. The filtrate was concentrated

in vacuo to give the crude diketopiperazine **297**. The pure product **297** was obtained as a white foam after column chromatography using a mixture of EtOAc and petroleum ether as eluent.

***tert*-butyl 3-(((3*R*,7*R*,8a*S*)-7-((tert-butoxycarbonyl)oxy)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indole-1-carboxylate 297**



Yield 52% over 2 steps (2.08 g); white foam; Rf = 0.20 (1/1 petroleum ether/EtOAc) ; m.p. 118-120 °C; **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.50 (9H, s, 3×C$\underline{H}_3$-23),1.67 (9H, s, 3×C$\underline{H}_3$-20), 2.02-2.12 (1H, m, C$\underline{H}_a$H$_b$-13), 2.49 (1H, dd, *J*=14.0 Hz, *J*=4.8 Hz, CH$_a\underline{H}_b$-13), 3.14 (1H, dd, *J*=14.5 Hz, *J*=8.5 Hz, C$\underline{H}_a$H$_b$-8), 3.29 (1H, dd, *J*=14.5 Hz, *J*=3.6 Hz, CH$_a\underline{H}_b$-8), 3.50 (1H, d, *J*=11.3 Hz, C$\underline{H}_a$H$_b$-15),3.93-4.04 (2H, m, CH$_a\underline{H}_b$-15, C$\underline{H}$-12), 4.23 (1H, ddd, *J*=8.5 Hz, *J*=3.6 Hz, *J*=3.6 Hz, C$\underline{H}$-9), 5.14 (1H, t, *J*=4.8 Hz, C$\underline{H}$-14), 6.07 (1H, d, *J*=3.6 Hz, N$\underline{H}$-10), 7.25 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-5), 7.34 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C$\underline{H}$-6), 7.48 (1H, s, C$\underline{H}$-2), 7.55 (1H, d, *J*=7.7 Hz, C$\underline{H}$-4), 8.16 (1H, d, *J*=7.7 Hz, C$\underline{H}$-7) ppm; **^{13}C NMR (100 MHz, $CDCl_3$) δ** 27.7 (3×CH_3-23), 28.2 (3×CH_3-20), 30.5 (CH_2-8), 35.6 (CH_2-13), 52.3 (CH_2-15), 56.4 (CH-12), 57.9 (CH-9), 72.7 (CH-14), 83.1 (C_q-22), 84.1 (C_q-19), 114.2 (C_q-3), 115.5 (CH-7), 118.8 (CH-4), 122.9 (CH-5), 125.0 (CH-6, CH-2), 129.4 (C_q-3a), 135.6 (C_q-7a), 149.4 (C=O-18), 152.4 (C=O-21), 165.1 (C=O-17), 168.1 (C=O-11) ppm; **IR** (cm^{-1}): υmax = 1733 (C=O), 1670 (C=O), 1451; **MS** (ES): *m/z* (%) = 344 (100) $[M + H]^+$, 522 (10) $[M + Na]^+$; **HRMS** (ESI): calcd. for $C_{26}H_{33}N_3NaO_7^+$ $[M + Na]^+$: 522.2211; found: 522.2220.

7.6.4. Selective synthesis of O-methylated cyclo(Trp, Hyp) 304

7.6.4.1. N-Boc protection of HypOMe·HCl 299

To a solution of 4-hydroxyproline methyl ester hydrochloride **299** (1 equiv., 24 mmol, 4.4 g) in acetone and water (3:2, 30 mL) were added triethylamine (2 equiv., 48 mmol, 6.8 mL), DMAP (0.05 equiv., 1.2 mmol, 147 mg) and $(Boc)_2O$ (1.4 equiv., 34 mmol, 7.4 g). The mixture was stirred overnight. The acetone was removed under reduced pressure and the residue was diluted with EtOAc (50 mL). The organic phase was subsequently washed with 0.5 M HCl (30 mL), water (30 mL) and brine (30 mL). Drying the organic phase over magnesium sulfate and concentration of the solvent under reduced pressure yielded the N-protected hydroxyproline methyl ester **300** as a colourless oil.[230] **Yield** 64% (3.93 g); colourless oil.

7.6.4.2. *O*-methylation of *N*-Boc-HypOMe 300

Next, N-protected hydroxyproline methyl ester **300** (1 equiv., 6.4 mmol, 1.58 g) was dissolved in dry DMF (10 mL) and cooled to 0 °C. Sodium hydride (1.25 equiv., 8.1 mmol, 0.32 g, 60% in mineral oil) and after 15 minutes methyl iodide (1.25 equiv., 8.1 mmol, 0.50 mL) were added at 0 °C. The mixture was allowed to warm up to room temperature and was monitored using HPLC-MS. To obtain full conversion, an extra portion of sodium hydride (0.5 equiv., 3.2 mmol, 0.13 g, 60% in mineral oil) and methyl iodide (1.0 equiv., 6.4 mmol, 0.40 mL) were added. After the careful addition of some droplets of water, EtOAc (25 mL) was added. The organic phase was washed with saturated aq. $NaHCO_3$ (20 mL) and 4 times with brine (4×20 mL). After drying over magnesium sulfate, the solution was concentrated under reduced pressure. The crude product was purified with column chromatography using a mixture of EtOAc and petroleum ether as eluent. The compound **301** was obtained as yellow oil. **Yield** 59% (0.98 g, *de* = 26%); yellow oil; R_f=0.56 (1/1 petroleum ether/EtOAc).

7.6.4.3. Boc deprotection from 301

The removal of the Boc protecting group was accomplished by dissolving compound **301** (1 equiv., 3.8 mmol, 0.98 g) in 4 M HCl in dioxane (65 mL).[231] After stirring the solution for 1.5 hour at room temperature, the conversion was complete. The solution was concentrated under reduced pressure providing the 4-methoxyproline methyl ester hydrochloride **302**. **Crude yield** quant. (0.74 g); yellow oil.

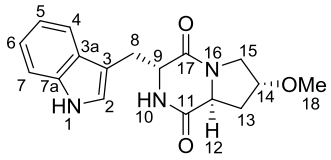
7.6.4.4. Dipeptide synthesis

4-Methoxyproline methyl ester hydrochloride **302** (1 equiv., 3.8 mmol, 0.74 g,) was dissolved in dry CH_2Cl_2 (50 mL) and *N*-benzyloxycarbonyltryptophan **155b** (1 equiv., 3.8 mmol, 1.28 g) and EDC·HCl (1 equiv., 0.73 g, 3.8 mmol) were subsequently added under a nitrogen atmosphere. The mixture was stirred at room temperature for 16 hours and was then washed three times with 1 M HCl (40 mL) and saturated aq. $NaHCO_3$ (40 mL). The organic layer was dried over magnesium sulfate and evaporated, yielding dipeptide **303**.

7.6.4.5. Hydrogenolysis and cyclization

To a solution of dipeptide **303** in MeOH (250 mL), 5 wt% of Pd/C was added. The reaction mixture was stirred under 5 atm of H_2 for 3 hours at room temperature. The Pd/C catalyst was removed by filtration through a celite pad. The filtrate was concentrated *in vacuo* to give the crude diketopiperazine. The pure product **304** was obtained after purification by reversed-phase chromatography (10 CVs 10% ACN, over 10 CVs to 20% ACN) as a white foam.

(3*R*,7*R*,8a*S*)-3-((1*H*-indol-3-yl)methyl)-7-methoxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione 304

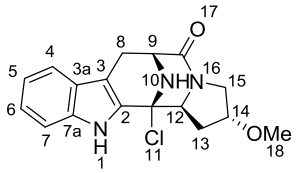


Yield 30% over 3 steps (0.54 g); colourless crystals; m.p. 94-98 °C; $[\alpha]_D^{25}$ = +40.0 (c=0.39 in $CHCl_3$); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 1.71-1.81 (1H, m, C<u>H</u>$_a$H$_b$-13), 2.26-2.33 (1H, m, CH$_a$<u>H</u>$_b$-13), 3.15 (3H, s, C<u>H</u>$_3$-18), 3.20-3.40 (4H, m, C<u>H</u>-12, C<u>H</u>$_2$-8, C<u>H</u>$_a$H$_b$-15), 3.65-3.72 (1H, m, CH$_a$<u>H</u>$_b$-15), 3.81-3.86 (1H, m, C<u>H</u>-14), 4.18-4.24 (1H, m, C<u>H</u>-9), 6.13 (1H, s, N<u>H</u>-10), 7.02 (1H, s, C<u>H</u>-2), 7.13 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C<u>H</u>-5), 7.20 (1H, dd, *J*=7.7 Hz, *J*=7.7 Hz, C<u>H</u>-6), 7.37 (1H, d, *J*=7.7 Hz, C<u>H</u>-7), 7.61 (1H, d, *J*=7.7 Hz, C<u>H</u>-4), 8.30 (1H, s, N<u>H</u>-1) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 30.6 (CH_2-8), 35.0 (CH_2-13), 51.0 (CH_2-15), 56.1 (CH_3-18), 56.2 (CH-12), 58.5 (CH-9), 76.2 (CH-14), 109.4 (C_q-3), 111.3 (CH-7), 118.8 (CH-4), 120.0 (CH-5), 122.5 (CH-6), 123.8 (CH-2), 126.9 (C_q-3a), 136.2 (C_q-7a), 165.5 ($C_{C=O}$-17), 169.4 ($C_{C=O}$-11) ppm; **IR** (cm^{-1}): υmax = 3237 (NH), 1643 (C=O), 1453; **MS** (ES): *m/z* (%): 314 (100) $[M+H]^+$; **HRMS** (ESI): calcd. for $C_{17}H_{20}N_3O_3^+$ $[M+H]^+$: 314.1499; found: 314.1514.

7.6.5. Introduction of the α-chloroamine

Diketopiperazine **304** (1 equiv., 3.0 mmol, 93.0 mg) was suspended in dry CH_2Cl_2 (3 mL) and cooled with an ice-bath to 0 °C under a nitrogen atmosphere. Diphosgene (3 equiv., 8.9 mmol, 0.11 mL), dissolved in dry CH_2Cl_2 (2 mL), was added dropwise to the suspension. The mixture was heated to reflux. After complete conversion (HPLC-MS) the organic phase was washed with saturated $NaHCO_3$ solution and with water. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. Purification of the residue by pHPLC using an isocratic H_2O/ACN gradient (30% ACN) provided the desired product **305** as a white foam.

(2*R*,6*R*,13*S*,13a*S*)-13-chloro-2-methoxy-1,2,3,6,7,12,13,13a-octahydro-5*H*-6,13-epiminopyrrolo-[1',2':1,2]azocino[4,5-b]indol-5-one 305



Yield 36% (35 mg); white foam; m.p. 236-238 °C; $[\alpha]_D^{25}$ = -160.7 (c=0.17 in $CHCl_3$); **^{1}H-NMR (400 MHz, $CDCl_3$) δ** 2.03 (1H, ddd, *J*=12.6 Hz, *J*=12.6 Hz, *J*=6.4 Hz, C<u>H</u>$_a$H$_b$-13), 2.24 (1H, dd, *J*=12.6 Hz, *J*=4.6 Hz, CH$_a$<u>H</u>$_b$-13), 2.65 (1H, s, N<u>H</u>-10), 2.94 (1H, dd, *J*=13.3 Hz, *J*=2.1 Hz, C<u>H</u>$_a$H$_b$-15), 3.03 (1H, d, *J*=16.4 Hz, C<u>H</u>$_a$H$_b$-8), 3.16 (1H, dd, *J*=16.4 Hz, *J*=6.4 Hz, CH$_a$<u>H</u>$_b$-8), 3.30 (3H, s, C<u>H</u>$_3$-18), 3.94-4.00 (1H, m, C<u>H</u>-14), 4.05 (1H, dd, *J*=12.6 Hz, *J*=4.6 Hz, C<u>H</u>-12), 4.21 (1H, d, *J*=6.4 Hz, C<u>H</u>-9), 4.37 (1H, dd, *J*=13.3 Hz, *J*=6.7 Hz, CH$_a$<u>H</u>$_b$-15), 7.13 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C<u>H</u>-5), 7.24 (1H, dd, *J*=7.6 Hz, *J*=7.6 Hz, C<u>H</u>-6), 7.36 (1H, d, *J*=7.6 Hz, C<u>H</u>-7), 7.47 (1H, d, *J*=7.6 Hz, C<u>H</u>-4), 8.27 (1H, s, N<u>H</u>-1) ppm; **^{13}C-NMR (100 MHz, $CDCl_3$) δ** 24.9 (CH_2-8), 35.5 (CH_2-13), 52.1 (CH_2-15), 56.7 (CH_3-18), 58.0 (CH-9), 66.5 (CH-12), 76.4 (C_q-11), 77.2 (CH-14), 109.2 (C_q-3), 111.6 (CH-7), 119.3 (CH-4), 120.4 (CH-5), 123.6

(CH-6), 126.5 (C_q-3a), 134.1 (C_q-2), 136.0 (C_q-7a), 169.1 (C=O-17) ppm; **IR** (cm^{-1}): υmax = 3273 (NH), 1638 (C=O), 1454; **MS** (ES): *m/z* (%): 332/334 (100/33) $[M + H]^+$; **HRMS** (ESI): calcd. for $C_{17}H_{20}ClN_3O_2^+$ $[M+H]^+$: 332.1160; found: 332.1169.

7.7. In vitro assays

7.7.1. Single-cell collagen invasion assay procedure cited from De Wever et al.[162]

The collagen invasion assays were assessed by the Laboratory of Experimental Cancer Research, Ghent University. Further details, critical points, timing, troubleshooting or suggestions can be found in the article from De Wever *et al*.

Preparation of a collagen gel:

1) Prepare collagen type I solution with a final concentration of 1 mg/mL collagen type I by mixing the following precooled (stored at 4 °C) components: 4 volumes collagen type I (stock is 3.49 mg/mL), 5 volumes of calcium- and magnesium-free Hank's balanced salt solution, 1 volume of minimal essential medium (10x), 1 volume of 0.25 M $NaHCO_3$, 2.65 volumes of standard medium and 0.3 volumes of 1 M NaOH to make the solution alkaline.
2) Add, for each test-condition, 1.25 mL of collagen type I solution to one well of a 6-well plate, spread homogeneously and let gelify on a flat surface in a humidified atmosphere of 10% CO_2 in air at 37 °C for at least 1 h to obtain a collagen gel with a 250 µm central thickness in the well.

Preparation of single-cells:

3) Prepare a single-cell suspension in standard medium by mild enzymatic dissociation, using a Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline wash followed by incubation with a trypsin/ethylenediaminetetraacetic acid solution, of an exponentially growing culture (usually 70% confluence is used).
4) Count a small aliquot of the cell suspension after staining with Trypan blue (0.04% Trypan blue in phosphate-buffered saline) to exclude dead cells.

Initiation of invasion model:

5) Prepare 1-2x105 viable, single-cells in 1 mL standard medium in 15 mL polypropylene tubes. Add test products such as growth factors (transforming growth factor-α) or drugs for preclinical (gefitinib) validation in desired concentration. Gently seed this mixture on top of blindcoded collagen type I gels.

6) Incubate cells in a humidified atmosphere with 10% CO_2 in air at 37 °C for 24 h.

Evaluation of single-cell invasion:

7) Focus an inverted phase-contrast microscope (with objective 10x or 20x) downwards from the culture medium to the top of the gel onto a single focal plane. The edges of the cells appear brighter (cells have a 'halo' of light) compared to the background. The degree of reduction in brightness depends on the refractive index. Dense structures such as the nucleus or fibrillar collagen appear dark. Cellular extensions invading the collagen matrix appear dark because they are located out of phase (focal plane). Occasionally and dependent from cell-line to cell-line whole single-cells have migrated into the gel and appear dark.
8) Take a digital image from 10-15 microscope fields.

Calculation of invasion index (manual cell counting):

9) Calculate the invasion index (cells with invasive extensions versus total number of cells x 100) by manual counting the number of invading and non-invading cells present in 10-15 microscope fields.

7.7.2. α2 (non-selective)

The binding activity towards the α2 receptor obtained from rat cerebral cortex, was assessed by CEREP (France).[221, 246] Radiolabeled [^{3}H]RX 821002 (0.5 nM) was used as ligand. Unlabeled RX 821002 was used as a reference compound (IC_{50} = 58.7 nM). Test compounds were incubated at room temperature for one hour. The decrease in binding of radiolabeled RX 821002 in the presence of the tested compounds is measured by scintillation counting.

7.7.3. D1 (antagonist radioligand)

The binding activity towards the D1 receptor of human origin, obtained by recombinant production in CHO cells, was assessed by CEREP (France).[247-248] Radiolabeled [^{3}H]SCH 23390 (0.3 nM) was used as ligand. Unlabeled SCH 23390 was used as a reference compound (IC_{50} = 0.242 nM). Test compounds were incubated at room temperature for a one hour period. The decrease in binding of radiolabeled SCH 23390 in the presence of the tested compounds is measured by scintillation counting.

7.7.4. N neuronal α7

The binding activity towards the N neuronal α7 receptor of human origin, obtained by recombinant production in SH-SY5Y cells, was assessed by CEREP (France).[249-250] Radiolabeled [^{125}I]α-bungarotoxin

(0.5 nM) was used as ligand. Unlabeled α-bungarotoxin was used as a reference compound (IC_{50} = 0.7 nM). Test compounds were incubated at 37 °C for 2 hours. The decrease in binding of radiolabeled α-bungarotoxin in the presence of the tested compounds is measured by scintillation counting.

7.7.5. N muscle-type (h) (antagonist radioligand)

The activity towards the N muscle-type receptor originating from TE671 cells was assessed by CEREP (France).[251-252] Radiolabeled [^{125}I]α-bungarotoxin (0.5 nM) was used as ligand. Unlabeled α-bungarotoxin was used as a reference compound (IC_{50} = 2 nM). Test compounds were incubated at room temperature for 2 hours. The decrease in binding of radiolabeled α-bungarotoxin in the presence of the tested compounds is measured by scintillation counting.

7.7.6. Serotonin 5-HT1 (non-selective)

The activity towards the serotonin 5-HT1 receptor originating from rat cerebral cortex was assessed by CEREP (France).[253-254] [^{3}H]Serotonin (2 nM) was used as ligand. Unlabeled serotonin was used as a reference compound (IC_{50} = 0.0011 µM). Test compounds were incubated at 37 °C for 10 min. The decrease in binding of radiolabeled serotonin in the presence of the tested compounds is reflected in the percentage inhibition of control specific binding measured by scintillation counting.

7.7.7. PDE5(h) (non-selective)

This assay was run by CEREP (France),[223, 255] using PDE5 from human platelets. [^{3}H]cGMP and cGMP (1 µM) were used as substrate. cGMP is broken down by the enzyme into guanosine-5'-triphosphate (GMP). The decrease in conversion of radiolabeled cGMP to GMP in the presence of the tested compounds is reflected in the percentage inhibition measured by scintillation counting. Incubation with the test compound was performed at room temperature over a one hour period. Dipyridamole served as a reference compound (IC_{50} = 0.7 µM).

7.7.8. Tubulin polymerization

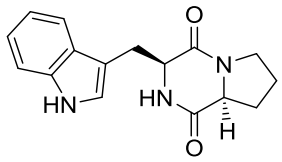
Microtubule polymerization was assessed by CEREP (France).[256-257] Tubulin protein was obtained from porcine brain. Test compounds were incubated at 37 °C for 15 min, in the presence of 1mM of guanosine-5'-triphosphate (GTP) as a tubulin polymerization inducer. The assembly of microtubule was followed using DAPI as a fluorescent probe. Vinblastine served as a reference compound (IC_{50} = 1.2 µM).

7.7.9. BCRP (h) inhibition

This assay was run by CEREP (France),[258-259] using BCRP of human origin, obtained by recombinant production in CHO-K1 cells. HOECHST 33342 (5 µM) was used as substrate and enzyme activity was determined by fluorimetry. Incubation with the test compound was performed at 37 °C, over a period of 20 minutes. KO143 served as a reference compound (IC_{50} = 480 nM).

VI. Summary

Brevianamide F or cyclo(L-Pro, L-Trp) **ia** is a fungal secondary metabolite that is made up of two amino acids, proline and tryptophan (Figure 34). It is also a basic skeleton which can be found in several other natural products such as tryprostatins A and B, fumitremorgin C and stephacidin A, which display a wide range of biological activities. These compounds are mostly isolated from *Streptomyces* and *Aspergillus* species. The goal of this dissertation was to synthesize a library of less complex, physiologically active analogues based on these natural compounds.

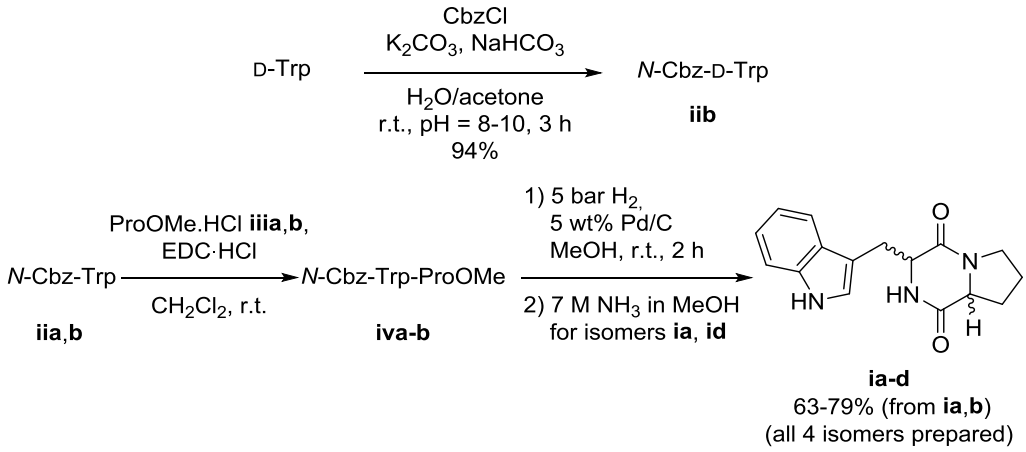


ia

Figure 34: Diketopiperazine cyclo(L-Trp, L-Pro) ia.

The first objective was to develop an efficient route for the synthesis of the basic skeleton, cyclo(Pro, Trp) **i**, starting from the L- and D-enantiomers of proline and tryptophan. Retention of the stereochemistry of these starting materials is essential. Different synthetic routes to obtain the desired compounds were evaluated. Most attention was paid to the coupling of the amino acids, but also the choice of the protecting group and the coupling order of the amino acids were examined. A first strategy was based on phosphorus-assisted amide formation and microwave heating. Next, benzotriazole activation was used, in which the amino acids were activated as their acylbenzotriazole counterparts to perform the coupling. A third strategy used 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) as a coupling reagent.

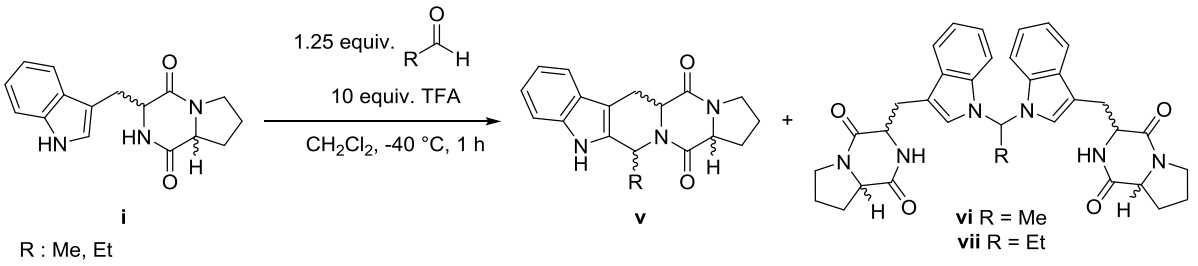
The phosphite-promoted condensation of unprotected amino acids only provided the homocoupling product of proline. The synthetic route based on the benzotriazole methodology required more steps than EDC mediated coupling, resulting in lower overall yields. The procedure with EDC·HCl appeared to be the best strategy for the coupling of the amino acids and was used to synthesize all four isomers in a limited number of steps and in good yields (Scheme 68). Spontaneous cyclization of the dipeptides towards the L,D and D,L isomers **ib** and **ic** occurred after Cbz-removal, but an extra cyclization step was required to obtain the *cis*-isomers **ia** and **id**.



Scheme 68: Synthesis of all isomers of cyclo(Trp, Pro) i.

Having the basic skeleton **i** in hand, the objective was to decorate this framework **i** with biologically relevant substituents to obtain a diverse set of functionalized brevianamide analogues. The main goal was to introduce a supplementary ring in the basic skeleton **i**, connecting the diketopiperazine ring with the indole moiety. The introduction of such an extra bridge leads to a higher conformational rigidity of the scaffold, which often results in increased receptor specificity. To achieve this, several modifications can be examined.

A first strategy that was attempted to achieve annulated derivatives was the Pictet-Spengler reaction. This reaction is usually performed on tryptamine or similar arylethylamines where the 'extra' ring is introduced first by the condensation with an aldehyde or a ketone. This 1,2,3,4-tetrahydro-β-carboline is then used as an intermediate that is modified to form a diketopiperazine. In the present work, the Pictet-Spengler reaction was performed on the available diketopiperazine **i**, which avoided the formation of different intermediates depending on the aldehyde used. A range of reaction conditions were tested but in all cases mixtures were obtained (Scheme 69). Based on HPLC-MS these mixtures presumably contained the desired Pictet-Spengler products **v**. However, these compounds could never be isolated. The only isolated products from these reaction mixtures are dimers **vi** and **vii**. Several new dimers of **i** that are linked by an alkyl bridge between the indole nitrogens were obtained under Pictet-Spengler conditions (Table 39).

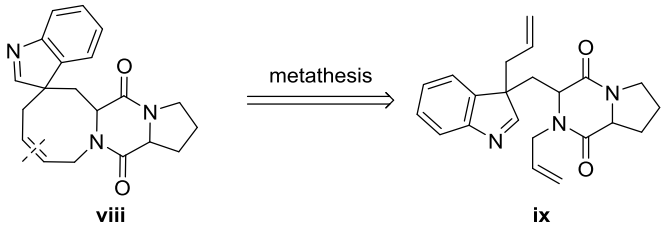


Scheme 69: Pictet-Spengler reaction of cyclo(Trp, Pro) i with an aldehyde.

Table 39: Isolated yields of the dimeric products.

Cpd	Starting compound	Yield (%)
via	Cyclo(L-Trp, L-Pro) **ia**	46%
vib	Cyclo(D-Trp, L-Pro) **ib**	32%
viia	Cyclo(L-Trp, L-Pro) **ia**	16%
viib	Cyclo(D-Trp, L-Pro) **ib**	12%

Another strategy to modify the diketopiperazine scaffold **i** was through alkylation using an unsaturated electrophile such as allyl bromide. These compounds might be suitable for further transformation by metathesis. The formation of a spiro-annulated derivative **viii** was aspired through metathesis of the diallylated piperazin-2,5-dione **ix** (Scheme 70).



Scheme 70: Aspired spiro-derivative viii *via* metathesis of diallylated ix.

The introduction of an allyl group at the indole C-3 in the *cis*-isomers **ia** and **id** using palladium catalysis succeeded, but concomitant ring formation took place yielding the allylated and annulated derivatives **xa** and **xb** (Figure 35). Their stereochemistry was confirmed using NOE experiments.

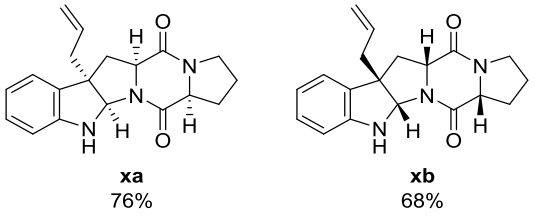
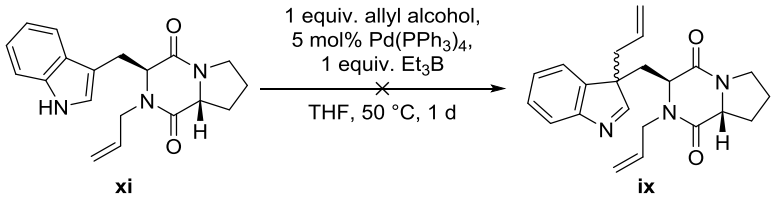


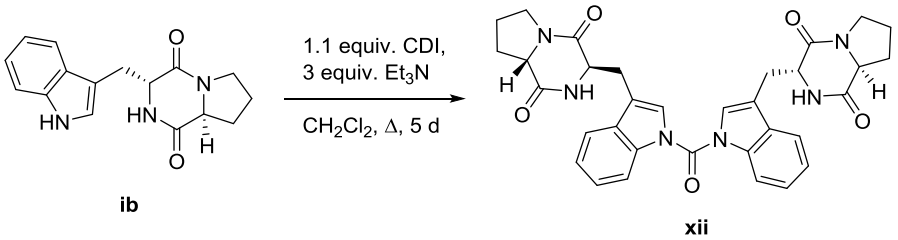
Figure 35: Allylated pentacyclic derivatives x.

Therefore, in our subsequent attempt the allyl group was first introduced at the amide nitrogen. To selectively introduce the allyl group at the amide nitrogen, several protecting and deprotecting steps at the indole nitrogen were necessary earlier in the synthesis. Unfortunately, epimerization took place during the synthesis of the mono-allylated product **xi**. Subsequent introduction of the allyl group at C-3 of the **xi** using the previously tested reaction conditions failed (Scheme 71).



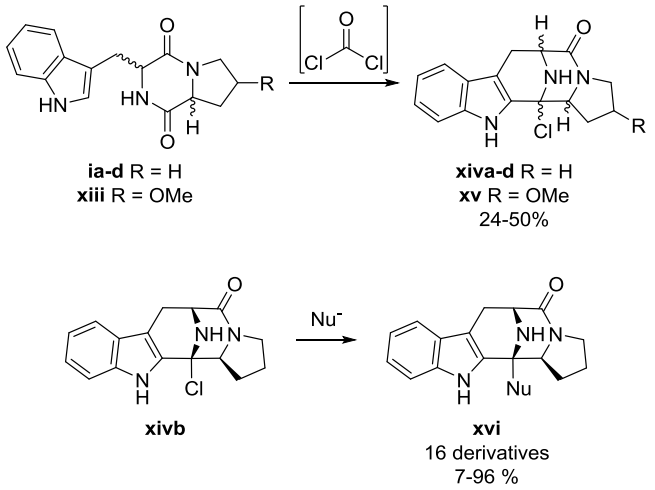
Scheme 71: Unsuccessful C-3 allylation of mono-allylated product xi.

The diketopiperazine scaffold **i** was also subjected to several difunctionalized electrophilic reagents to accomplish the formation of an extra ring by forming a bridge between the amide nitrogen and the indole moiety. Only the reaction with CDI (1,1'-carbonylbis-1*H*-imidazole) gave a (clean) conversion and resulted in the formation of a dimer **xii** (Scheme 72).



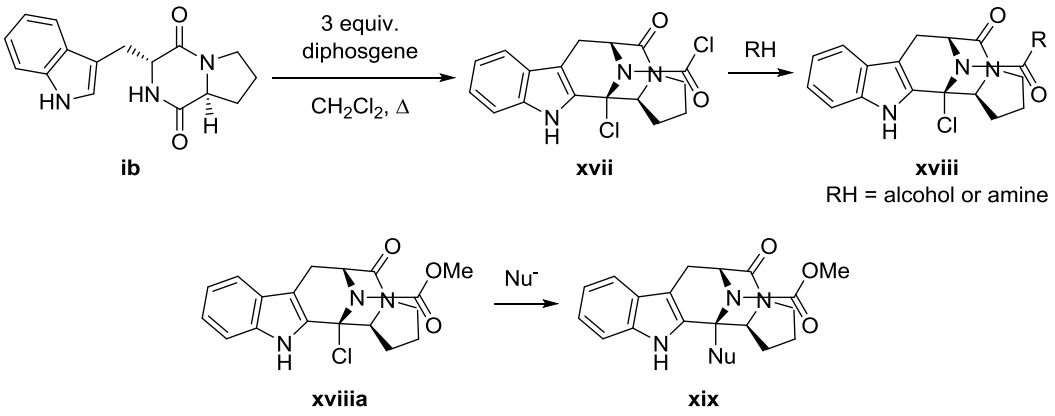
Scheme 72: Dimer xii from ib obtained with CDI.

Another electrophile that was evaluated is phosgene, in the form of tri- or diphosgene. This resulted in the formation of pentacyclic compounds **xiv** and **xv** containing an α-chloroamine. Density functional theory (DFT) calculations suggest that the α-chloroamine is formed by direct attack by the C-2 atom of the indole group and not by C-3 attack and a subsequent 1,2-shift. The purification of **xiv** proved to be difficult and an optimization of the reaction conditions was performed. Modification of this rigid 3,5-bridged piperazin-2-one **xivb** was possible by substitution of the chlorine atom in **xivb**, which offers a new avenue towards synthetic analogues of brevianamides, fumitremorgins and (spiro)tryprostatins. A range of O-, N-, S- and C-nucleophiles (Nu^-) was introduced under basic conditions, affording compounds **xvi** (Scheme 73). The purification of these derivatives **xvi** was troublesome, resulting in low isolated yields. A preliminary bioactivity screening of selected compounds revealed that the novel pentacyclic derivatives **xvi** possess significant breast cancer resistance protein (BCRP) inhibition.



Scheme 73: Synthesis of α-chloroamines xiv-xv and their derivatives xvi.

Additionally, the carbamoyl chloride **xvii** was obtained when the pentacyclic **xiv** was left to react with diphosgene for a longer reaction time. This carbamoyl chloride **xvii** could easily be converted into the corresponding carbamate or urea **xviii**, by reaction with an alcohol or an amine, respectively. On their turn, these compounds could be modified by substitution of the chlorine atom, affording derivatives **xix** (Scheme 74).

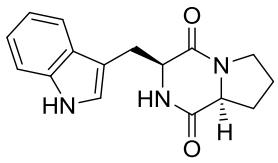


Scheme 74: Synthesis of carbamate and urea derivatives xviii. Further substitution of the chlorine atom yields compounds xix.

In conclusion, a novel class of brevianamide F analogues, which possess interesting BCRP inhibitory activity, was synthesized. The presence of the α-chloroamine functionality provides an easy way to modify these 3,5-bridged structures, which may yield derivatives with a better BCRP inhibitory activity.

VII. Samenvatting

Brevianamide F of cyclo(L-Pro, L-Trp) **ia** is een secundair metaboliet van fungale oorsprong en is opgebouwd uit de aminozuren proline en tryptofaan (Figuur 1). Het vormt eveneens het basisskelet van verschillende andere natuurproducten zoals tryprostatines A en B, fumitremorgine C en stefacidine A, die een brede waaier aan biologische activiteiten vertonen. Deze verbindingen worden meestal geïsoleerd uit *Streptomyces* en *Aspergillus* species. Het doel van dit doctoraat bestond erin om een bibliotheek aan minder complexe, maar toch fysiologisch actieve verbindingen te synthetiseren die gebaseerd zijn op deze natuurproducten.

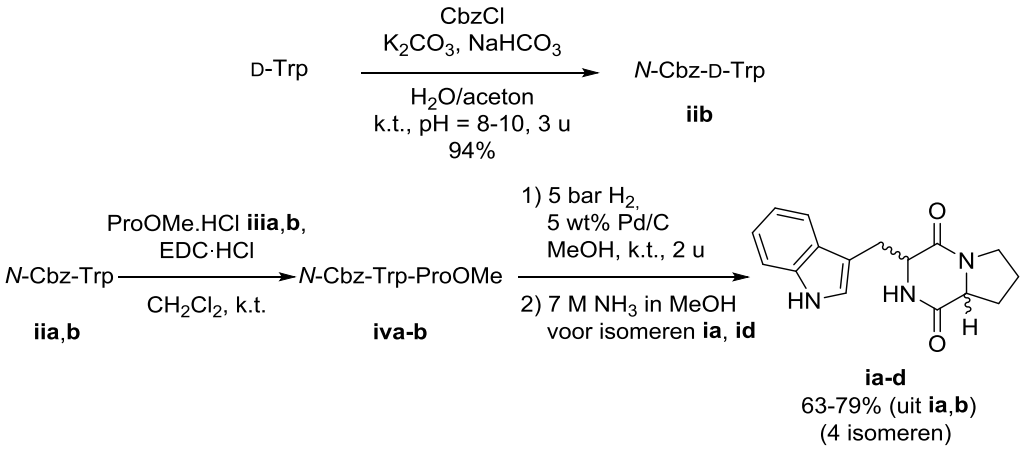


ia

Figuur 1: Diketopiperazine cyclo(L-Trp, L-Pro) ia.

De eerste doelstelling was om een efficiënte route te ontwikkelen voor de synthese van het basisskelet cyclo(Pro, Trp) **i**, uitgaande van de verschillende isomeren van proline en tryptofaan. Hierbij is de retentie van de stereochemie van de startproducten van essentieel belang. Verschillende syntheseroutes om de gewenste verbindingen te bekomen werden vergeleken. De meeste aandacht werd geschonken aan de koppeling van de aminozuren, maar ook de invloed van de beschermende groepen en van de volgorde waarin de aminozuren werden gekoppeld, werden onderzocht. Een eerste strategie was gebaseerd op fosforgeassisteerde amidevorming en microgolfverwarming. Vervolgens werd de benzotriazoolmethodologie gebruikt, waarbij de aminozuren werden geactiveerd als hun overeenkomstige acylbenzotriazolen om de koppeling te bewerkstelligen. Een alternatieve strategie maakte gebruik van EDC·HCl als koppelingsreagens.

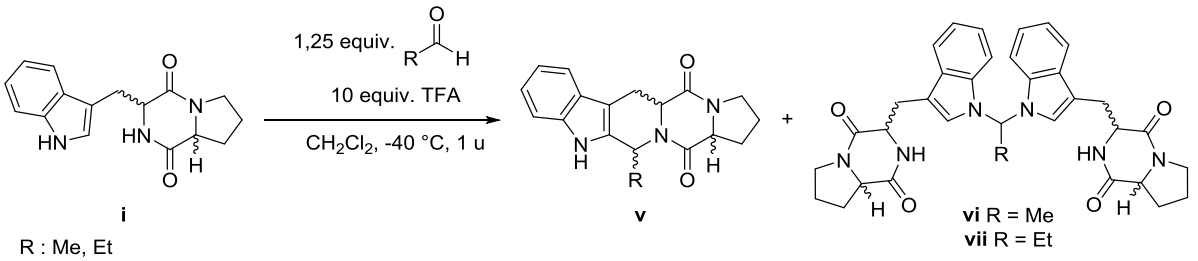
De fosfietgemedieerde condensatie van onbeschermde aminozuren leverde enkel het homogekoppelde product afgeleid van proline. De syntheseroute gebaseerd op de benzotriazoolmethodologie vereiste beduidend meer stappen dan de EDC-geassisteerde koppeling, wat resulteerde in lagere rendementen. De procedure met EDC·HCl bleek de beste strategie te zijn voor de koppeling van de aminozuren, en werd gebruikt om de vier isomeren te synthetiseren in een beperkt aantal stappen en met goede rendementen (Schema 1). Er vond een spontane ringsluiting plaats van de dipeptiden bekomen na ontscherming van de Cbz-groep, met vorming van de L,D en D,L isomeren **ib** en **ic**. Een extra stap was echter nodig om de ringsluiting te bewerkstelligen bij de *cis*-isomeren **ia** en **id**.



Schema 1: Synthese van alle isomeren van cyclo(Trp, Pro) i.

Met het skelet **i** ter beschikking, was het doel vervolgens om deze basisstructuur **i** te decoreren met relevante substituenten, teneinde op die manier een gevarieerde reeks aan gefunctionaliseerde analogen te verkrijgen. De hoofddoelstelling bestond uit het introduceren van een extra ring in het basisskelet **i**, die een verbinding maakt tussen de diketopiperazinering en de indoolgroep. Het invoegen van een extra brug leidt tot een grotere conformationele rigiditeit van het skelet, wat vaak aanleiding geeft tot een toegenomen selectiviteit voor een receptor. Om deze doelstelling te bereiken werden verschillende modificaties onderzocht.

Een eerste strategie die werd getest, bestond erin om geannuleerde derivaten te bekomen door middel van een Pictet-Spenglerreactie. Deze reactie wordt doorgaans uitgevoerd op tryptamine of gelijkaardige arylethylamines, waarbij de 'extra' ring eerst wordt geïntroduceerd door de condensatie met een aldehyde of een keton. Dit 1,2,3,4-tetrahydro-β-carboline kan dan als intermediair dienen dat verder wordt omgezet in een diketopiperazine **i**. De Pictet-Spengler reactie werd in dit werk echter uitgevoerd op het reeds gevormde diketopiperazine **i**, waardoor de nood aan verschillende intermediairen, afhankelijk van het gebruikte aldehyde, werd vermeden. Verschillende reactiecondities werden onderzocht, maar keer op keer werden mengsels bekomen (Schema 2). Op basis van HPLC-MS-analyse bevatten deze mengsels wel degelijk het beoogde Pictet-Spengler product **v**. Deze verbindigen konden echter nooit geïsoleerd worden. De enige producten die werden bekomen uit deze reactiemengsels zijn de dimeren **vi** en **vii**. Verschillende nieuwe dimeren van **i**, die een alkylbrug bevatten tussen de indoolstikstofatomen, werden verkregen gebruik makende van reactiecondities die typisch zijn voor een Pictet-Spenglerreactie (Tabel 1).

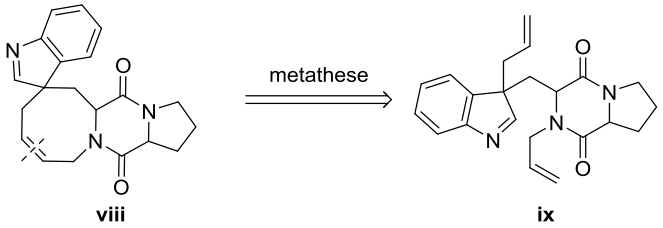


Schema 2: Pictet-Spenglerreactie van cyclo(Trp, Pro) i met een aldehyde.

Tabel 1: De rendementen van de opgezuiverde dimere producten.

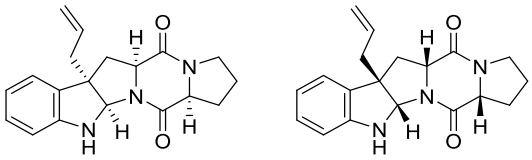
Verbinding	Startverbinding	Rendement(%)
via	Cyclo(L-Trp, L-Pro) **ia**	46%
vib	Cyclo(D-Trp, L-Pro) **ib**	32%
viia	Cyclo(L-Trp, L-Pro) **ia**	16%
viib	Cyclo(D-Trp, L-Pro) **ib**	12%

Een andere strategie om het diketopiperazineskelet **i** te wijzigen bestond uit een alkylering door middel van onverzadigde elektrofielen zoals allylbromide. Deze verbindingen zouden geschikte substraten moeten zijn voor een verdere omzetting door middel van metathese. De vorming van een spirogeannuleerd derivaat **viii** werd beoogd door middel van metathese van het digeallyleerde piperazin-2,5-dion **ix** (Schema 3).



Schema 3: Beoogde spiro-derivaten viii met behulp van metathese van het digeallyleerd ix.

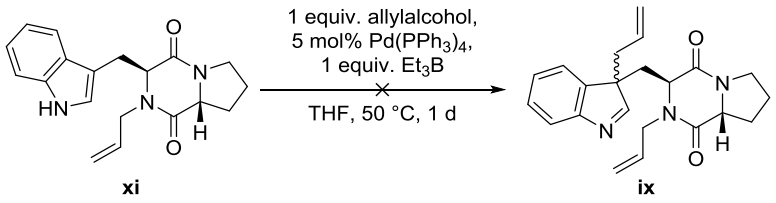
Het invoeren van een allylgroep op C-3 van de indoolgroep in *cis*-isomeren **ia** en **id** gebruik makende van palladiumkatalyse was succesvol, maar tegelijkertijd vond een ringsluiting plaats die de geallyleerde en ringgesloten derivaten **xa** en **xb** opleverde (Figuur 2). De stereochemie van deze verbindingen werd bevestigd met behulp van NOE experimenten.



xa 76% **xb** 68%

Figuur 2: Geallyleerde pentacyclische derivaten x.

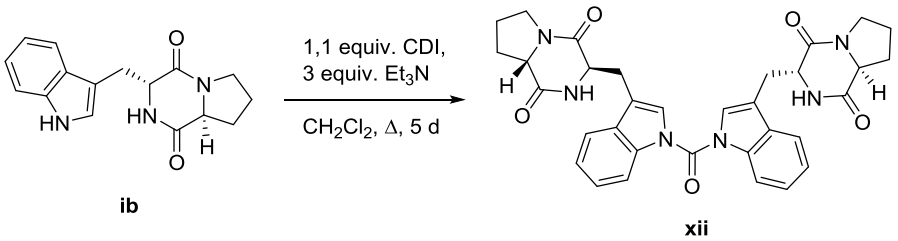
Om deze ringsluiting te voorkomen werd de allylgroep eerst ingevoerd op het amidestikstofatoom. Om de allylgroep selectief op de amidestikstof te introduceren waren eerder in de synthesesequentie verschillende beschermings- en ontschermingsstappen nodig. Er werd echter epimerisatie vastgesteld tijdens de synthese van het monogeallyleerde product **xi**. De daaropvolgende invoering van de allylgroep op C-3 van **xi**, gebruik makende van de eerder geteste reactiecondities, faalde echter (Schema 4).



xi **ix**

Schema 4: Gefaalde C-3 allylering van het monogeallyleerd product xi.

Het diketopiperazineskelet **i** werd eveneens behandeld met verschillende digefunctionaliseerde elektrofiele reagentia om de vorming van een extra ring, door middel van een brug tussen het amidestikstofatoom en de indoolgroep te bewerkstelligen. De reactie met CDI gaf conversie met vorming van slechts één product, opnieuw een dimeer **xii** (Schema 5).

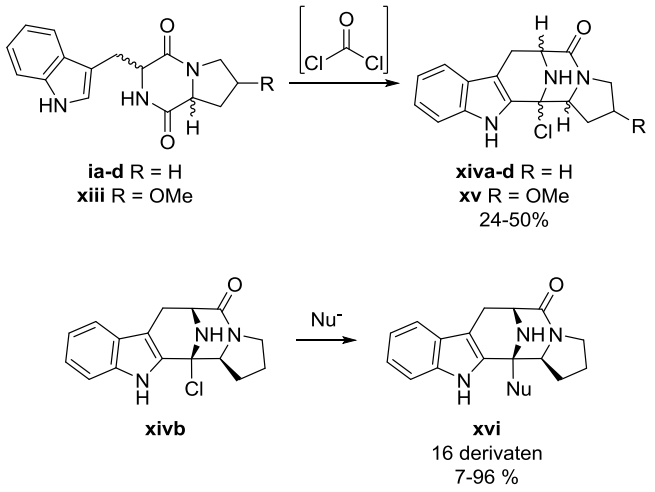


ib **xii**

Schema 5: Dimeer xii van ib verkregen door middel van CDI.

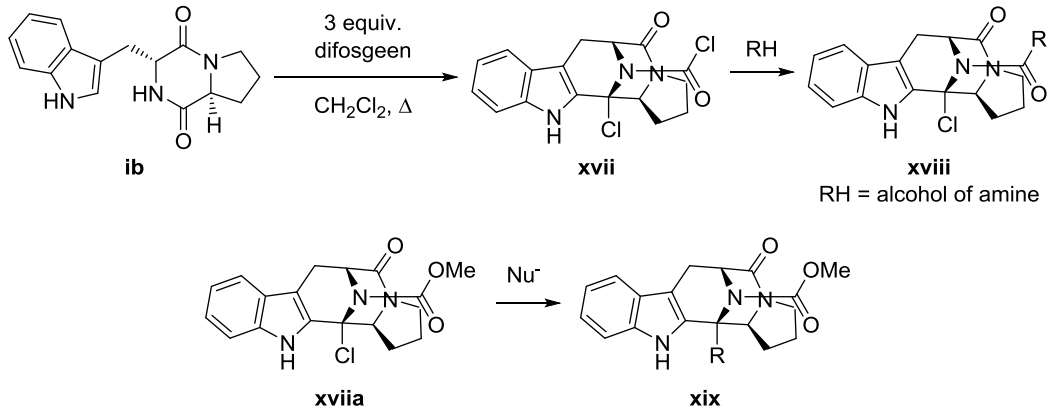
Een ander elektrofiel dat eveneens werd geëvalueerd was fosgeen, in de vorm van tri- of difosgeen. Hier werden pentacyclische verbindingen **xiv** en **xv** bekomen, die een α-chlooramine bevatten. 'Density functional theory' (DFT) berekeningen suggereren dat het α-chlooramine wordt gevormd door directe aanval van het C-2 atoom van de indoolgroep, en niet door C-3-aanval en daaropvolgende 1,2-shift. De isolatie van **xiv** verliep moeizaam en een optimalisatie van de

reactiecondities werd uitgevoerd. Het modificeren van het rigide 3,5-gebrugde piperazin-2-on **xiv** was mogelijk door middel van substitutie van het chlooratoom. Dit creëert een nieuwe mogelijkheid voor het bereiden van synthetische analogen van de brevianamides, fumitremorgines en (spiro)tryprostatines. Een gamma aan O-, N-, S- en C-nucleofielen (Nu^-) werden ingevoerd onder basische omstandigheden, met substitutie van het chlooratoom op het bruggenhoofd van **xivb**. Dit resulteerde in een reeks derivaten **xvi** (Schema 6). De isolatie van deze producten **xvi** verliep eveneens moeizaam, wat resulteerde in lage rendementen. Een eerste screening van de biologische activiteit van enkele verbindingen toonde aan dat deze nieuwe pentacyclische derivaten **xvi** een significante inhibitie uitoefenen van het 'Breast Cancer Resistance Protein' (BCRP).



Schema 6: Synthese van α-chloroamines xiv-xv en verdere derivaten xvi.

Daarenboven werd het carbamoylchloride van **xvii** bekomen wanneer **ib** lang genoeg werd gereageerd met difosgeen. Dit carbamoylchloride **xvii** kon gemakkelijk omgezet worden in het overeenkomstige carbamaat of ureum **xviii**, respectievelijk door reactie met een alcohol of een amine. Deze verbindingen konden op hun beurt verder gemodificeerd worden door substitutie van het chlooratoom, met vorming van derivaten **xix** (Schema 7).



Schema 7: Synthese van carbamaten en urea xviii. Verdere substitutie van het chlooratoom levert verbindingen xix.

Samengevat, werd een nieuwe klasse van brevianamide F-analogen ontwikkeld die een inhiberende werking uitoefenen op BCRP. De aanwezigheid van het α-chlooramine maakt de synthese van een reeks aan derivaten mogelijk teneinde een betere BCRP-inhiberende activiteit te bekomen.

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Curriculum vitae

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EDUCATION

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2009-2011 Master in Bioscience Engineering: Chemistry & Bioprocess Technology (graduated with greatest distinction)

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2009 Internship at Vandemoortele (Izegem, July-August)

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SCIENTIFIC PUBLICATIONS

INTERNATIONAL PEER-REVIEWED JOURNALS (A1)

Wauters, I.; Goossens, H.; Delbeke, E.; Muylaert, K.; Roman, B. I.; Van Hecke, K.; Van Speybroeck, V.; Stevens, C. V., Beyond the diketopiperazine family with alternatively bridged brevianamide F analogues. *J. Org. Chem*. **2015**, *80*, 8046-8054.

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14th Belgian Organic Synthesis Symposium (BOSS XIV), July 13-18, 2014, Louvain-La-Neuve, Belgium.
Poster presentation: **Wauters, I.**; Delbeke, E.; Heugebaert, T. S. A.; Roman, B. I.; Van Hecke, K.; Stevens, C. V., Cyclization of cyclo(Trp, Pro) toward 3,5-bridged piperazin-2-ones.

The 2014 Belgian Peptide Group Meeting, February 10-11, 2014, Ghent, Belgium.
Poster presentation: **Wauters, I.**; Delbeke, E.; Heugebaert, T. S. A.; Roman, B. I.; Van Hecke, K.; Stevens, C. V., Cyclization of cyclo(Trp, Pro) toward 3,5-bridged piperazin-2-ones.

25th International Congress of the International Society of Heterocyclic Chemistry (ISHC), August 23-28, 2015, Santa Barbara, United States of America.
Oral presentation: Stevens, C. V.; Heugebaert, T. S. A.; Van Overtveldt, M.; **Wauters, I.**, Synthesis of azabicyclic natural product analogues aiming at biological activity.